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EXCHANGE OF SUBSTANCES IN AQUEOUS SOLUTION BETWEEN JOINTS AND THE VASCULAR SYSTEM^{1 2}

By FREDERIC W. RHINELANDER, 2d, GRANVILLE A. BENNETT, AND
WALTER BAUER

(From the Departments of Pathology and Medicine, Harvard Medical School, the Medical Clinic of the Massachusetts General Hospital and the Massachusetts Department of Public Health, Boston)

(Received for publication June 20, 1938)

In experiments previously described (1) it was shown that materials of colloidal dimensions as the proteins of egg white and horse serum were removed from joints only by way of the lymphatics. The removal of such proteins was hastened by passive exercise of the joint.

The present series of experiments was undertaken to examine the manner of removal of materials of small molecular dimensions present in homogeneous aqueous solutions from normal joints and from joints in certain induced pathological conditions. This series is part of the study to increase our knowledge of normal joint physiology and of the factors involved in the production and maintenance of joint effusions.

Solutions containing a drug having a powerful effect on the blood pressure were selected because they offered a ready and continuous means of observing their transference from joint cavity to blood stream. Preliminary experiments had shown mecholyl (acetyl beta methyleholine chloride, Merck), a potent and evanescent vasodepressor, relatively stable among choline compounds to be the most suitable drug for the purposes of the experiment. Therefore mecholyl was used more extensively than any other, although adrenalin, pituitrin, and pilocarpine were also employed. One series of normal joints was studied at rest and under passive exercise. The investigation was then extended to joints in various abnormal states.

PROCEDURE

Knee joints of cats were used in all experiments. The cats were anesthetized intraperitoneally with nembutal, dial or with a combination of both. The most satisfactory steady circulatory state was obtained with dial 4.06 cc. per kgm., followed in 15 minutes by nembutal 20 mgm. per kgm.

A cannula was placed in the common carotid artery for recording the blood pressure on a slowly moving smoked drum. A second cannula bearing a short length of rubber tubing was fixed in the axillary vein. Intravenous injections were given by syringe, with the needle inserted into this tubing and were washed into the vein on each occasion by 1 cc. of isotonic saline delivered by pipette.

Injection of a dye into the knee joints of recently killed cats had shown that at least 1 cc. of solution could be given without escape into the structures surrounding the joint. During experimental procedures intra-articular injections were made in 0.25 cc. of solution. Control experiments showed that mecholyl of varying strength used for such injections did not lose its potency under the conditions employed.

Exercise of a joint was carried out mechanically by flexion and extension at the rate of 60 times per minute.

At the end of each experiment, the amount of drug remaining in the joint was determined in the following manner. The joint was aspirated by means of a needle and close fitting syringe. The few drops of synovial fluid thus obtained were placed in a small wide test tube. The joint was then washed out with 1 cc. of normal saline, with the same syringe and without removing the needle from the joint, and the washings were added to the fluid first aspirated. This washing-out process was repeated once. The fluid first aspirated and the joint and test tube washings, mixed together, were then injected intravenously into the animal and the effect on the blood pressure recorded. An attempt was then made to match, by means of a known intravenous dose of mecholyl, the tracing which had thus been obtained, giving thereby an approximate estimate of the amount of drug which had been recovered from the joint. Preliminary experiments had indicated that by the above

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² Presented before the American Society for Clinical Investigation at Atlantic City, New Jersey, May 1936.

* A "solution of dial with urethane" supplied by the Ciba Company.

Cat Number 25

mod of washing out joints, better than 90 per cent previously injected mecholyl could be recovered. This deemed sufficiently accurate for the purpose of these experiments.

Accuracy of assay of the washings was sacrificed to simplicity of procedure, to assure that the cat remained in a steady state as possible for the injection of both joints. Comparison was made of the choline-compound content of each joint. It was sufficient to establish by means of the washings that one joint contained definitely more or less residual choline-compound than the other, that both contained approximately the same. In analyzing the results of an experiment, the point of maximal effect on each blood pressure tracing was measured, and these were compared.

No joint experiment was undertaken until repeated intravenous injections of a standard dose of mecholyl (0.0005 mgm) gave constant effects, thus indicating that the cat was in a steady state. This same standard intravenous dose, or one of about the same magnitude, even at the end of the experiment, served to indicate whether or not the circulatory state had altered.

A brief description of a typical experiment with the results obtained is given below.

After the usual preparation, a standard intravenous dose of mecholyl (0.0005 mgm) was given 4 times, with an average blood pressure effect of 48 mm Hg. The cat appeared to be in a steady state.

The right knee joint was injected with mecholyl 0.0025 mgm, and kept at rest. The resulting fall of blood pressure reached a maximum of 31 mm Hg one minute three seconds after the injection (Figure 1). When the drug had remained in the joint for 6 minutes, the joint was washed out. Immediate intravenous injection of the joint washings produced a 50 mm drop of blood pressure.

The left knee joint was then injected with the same dose of mecholyl and exercised for 6 minutes. The resulting fall of blood pressure reached a maximum of 45 mm Hg, 42 seconds after the injection. At the end of 6 minutes, the joint was washed out. The washings given at once intravenously caused a blood pressure drop of 40 mm Hg.

Assay of washings. The initial effect of the standard intravenous dose of mecholyl had approximated the effect of the washings from the resting joint (48 and 50

25
RESTING

NORMAL JOINTS

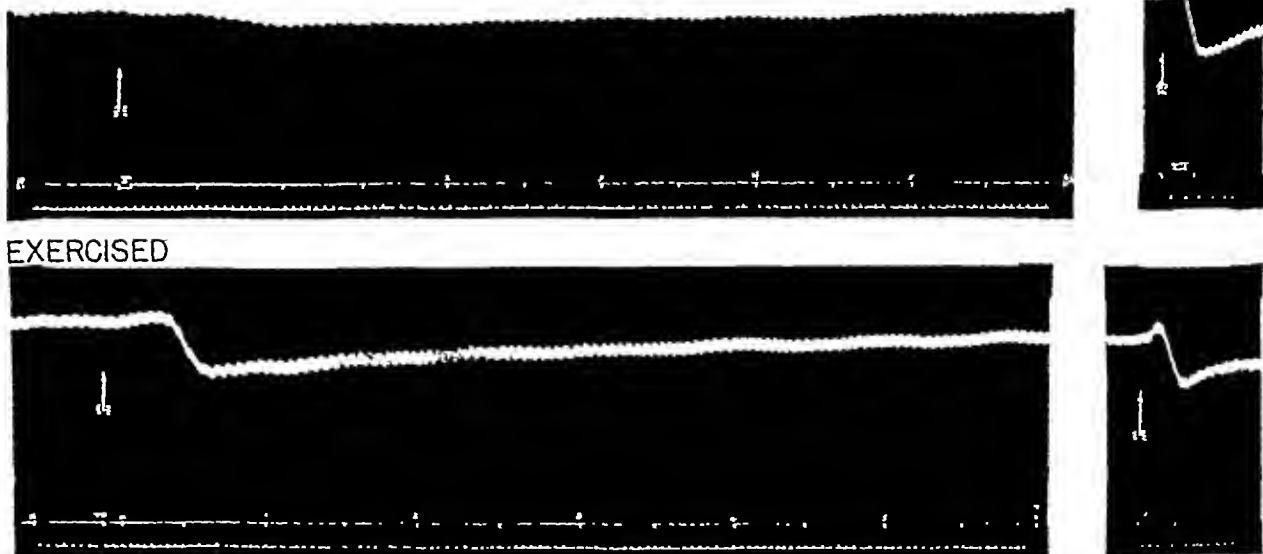


FIG 1 CAT 25—MECHOLYL (0.0025 mgm) INJECTED INTO EACH JOINT

Left-hand tracings show greater blood pressure effect with exercise. Right-hand tracings show less residual mecholyl in the washings from the exercised joint.

In all figures, the tracings represent arterial blood pressure. The arrows indicate the time of intra-articular or intravenous injection. The timer records 3-second intervals. The number of minutes after injection is also indicated in some instances.

In Figures 1, 2, and 3, the left-hand tracings show the result of the intra-articular injection of mecholyl, the right-hand tracings show the effect of the intravenous injection of the respective joint washings.

mm. Hg, respectively), so no further assay was necessary in this case. In an attempt to approximate the effect of the washings from the exercised joint, mecholyl 0.0004 mgm. was given intravenously, leading to a blood pressure drop of 42 mm. Hg (as against 40 mm. Hg for the washings)

Results There was a greater initial blood pressure effect in the experiment with the exercised joint, and the washings from this joint contained less residual mecholyl, indicating that more had been absorbed. (Compare with Table I for manner of expressing these data in brief form)

REMOVAL OF MECHOLYL FROM NORMAL JOINTS

The removal of mecholyl from joints, normal except for the injection of the drug in 0.25 cc. of distilled water, was studied in experiments on 18 cats. Doses varying from 0.00025 to 0.01 mgm were used

That absorption from the joint cavity took place was shown either directly by an effect on the blood pressure of the cat soon after the injection into the joint (with the larger doses), or indirectly by failure on washing out the joint to recover the full amount of drug which had been injected (with the smaller doses). In Table I will be found the results of the experiments on normal joints

It will be seen from this table that the variability of the effect obtained from the same dose, whether injected into a joint or a vein, bears no relation to the weights of the cats. The apparent differences in sensitivity to mecholyl of the various cats would thus appear to be one factor in the inconstant effects of the intra articular injections, therefore no conclusions relative to small differences in permeability of the synovial membrane in different experiments can be drawn from these data

Analysis of the complete data obtained in this group of experiments brings out the following major points of significance

1 Mecholyl in aqueous solution was readily absorbed from all joints without significant exception

If the absorption of mecholyl resulted in a direct effect on the blood pressure, it always did so within narrow time limits—approximately 30 to 60 seconds after injection. Absorption of the smallest doses (from 15 of 34 joints) was insufficient to produce a definite effect on the blood pressure. That this could occur, with

mecholyl actually reaching the blood stream, was proved with Cat 17. The amount of mecholyl which had been absorbed from one of the joints of this cat was determined as accurately as possible by a very close assay of the washings. This amount of mecholyl was then run into the cat intravenously, by means of a constant drip apparatus, at a rate calculated to cover the same period of time that the drug had remained inside the joint. At no time during the infusion of mecholyl at this rate was any effect on the blood pressure obtained. Increasing the rate, with this and other cats, led to a slow sequence of transitory effects on the blood pressure (typical of mecholyl effects) which occurred presumably as the concentration of the drug in the blood repeatedly reached the liminal level. That, with all cats, only a single and relatively large blood pressure effect was ever obtained after *intra-articular* injection suggests that the rate of absorption of mecholyl from normal joints declines from the initial, rapid rate

2 Exercise increased strikingly the rate of absorption of mecholyl from a normal joint

This was clearly demonstrated in 8 of the 9 experiments set up to test the point—Cats 12, 14, 15, 22, 25, 26, 27, 29 (See Figure 1). With Cats 15 and 22 this difference between the resting and the exercised joint may not be at once obvious. Although at the end of each experiment on the joints of Cat 15 equal amounts of drug had been absorbed, the drug had been longer in that joint which had not been exercised. Cat 22 was slightly more sensitive to mecholyl in the experiment with exercise, making the recorded effect of the washings greater than it should have been in comparison with the washings from the unexercised joint

3 The longer mecholyl remained in a joint, the more of it was absorbed

This applies only to joints in the same status regarding rest or exercise. Exercise was more potent than time in promoting absorption. However, there were three experiments in which an unusually great absorption occurred for the short time that the drug remained in the joints—both joints of Cat 12, injected with the very large dose of 0.01 mgm., and the single successfully injected joint of Cat 18, where the usual $\frac{1}{2}$ of 0.0025 mgm was a

washing When both joints were rested, any possible difference between the normal and abnormal joints was too small to be detected

In this group of experiments on washing out synovial fluid, it would be expected that depletion of synovial fluid would inhibit egress of water from the joint. But this would not prevent the rapid transference of foreign compounds (as of the drug mecholyl) from the joint cavity to the blood stream. (See experiment with pilocarpine below, where an effusion was being formed while the drug was being absorbed.) Thus the chief abnormal factor affecting the absorption of mecholyl in these experiments would appear to have been the mild irritation caused by the saline used to wash out the synovial fluid. Consequently the results in this group of experiments must be interpreted in conjunction with those of the succeeding group

B Mild inflammation produced by saline injection

Since previous studies with other animals had shown that injection of normal saline into joints produced a mild inflammation, with a definite rise of the synovial fluid cell count (2), this method was adopted in a series of 9 cats. One or both joints of these cats were injected with 1 cc of sterile saline, under light ether anesthesia, and the saline was left in the joint. After 4 such injections, at intervals of 3 or 4 days, the usual mecholyl experiment was carried out on each joint, with exercise or rest for 10 minutes.

The results in this series, shown in Table IV, were essentially similar to those in the preceding

series where the synovial fluid was simply washed out with saline, except that the tendency for more mecholyl to be absorbed from the abnormal joint at rest was slightly more pronounced (Figure 2)

From the above two groups of experiments, on washing out the synovial fluid with saline and on injecting saline, the following may be added to the significant observations on the removal of mecholyl from joints

4 Mild acute inflammation led to slightly increased absorption of mecholyl from joints, more definitely with exercise than with rest

C Severe inflammation produced by aleuronat injection

Having observed previously that the injection of aleuronat⁵ caused marked inflammation of the synovial membrane, with fibrinous and cellular exudation (2), this substance was employed in the final series of abnormal joints. The sterile aleuronat suspension was injected, under ether anesthesia, into one or both joints of 10 cats on two occasions, one and two weeks before the joints were used for experiments identical with those of the saline-injection series. The striking results are exhibited in Table V. (See also Figure 3)

From these results the following observation is justified

5 Severe acute inflammation led to definitely increased absorption of an aqueous solution of mecholyl from both exercised and resting joints

⁵ A suspension of 5 per cent aleuronat and 3 per cent starch in 0.5 per cent saline.

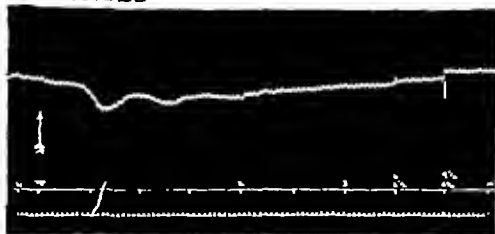
TABLE IV*
Joints mildly inflamed by injections with saline on three previous occasions

One joint injected with saline, both joints rested					One joint injected with saline, both joints exercised					Both joints injected with saline, one joint exercised				
Cat num- ber	Greater blood pressure effect		Greater absorption		Cat num- ber	Greater blood pressure effect		Greater absorption		Cat num- ber	Greater blood pressure effect		Greater absorption	
	Normal joint	Ab- normal joint	Normal joint	Ab- normal joint		Normal joint	Ab- normal joint	Normal joint	Ab- normal joint		Resting joint	Exercised joint	Resting joint	Exercised joint
35		++++	++	++	46		++++		++++	62	++			++++
45		++	++	++	65		++		++	63	++			++++
47	=	=	=	=	66	=	+		+	64		++++		++++

* Mecholyl 0.003 mgm. was injected into each knee joint of each cat in each experiment. The magnitude of observed difference in blood pressure effect, or in absorption, with respect to one of the two joints of a cat, is indicated by the number of +'s. The symbol "=" indicates that the results were the same for the two joints.

64
EXERCISED

TREATED JOINTS



RESTING

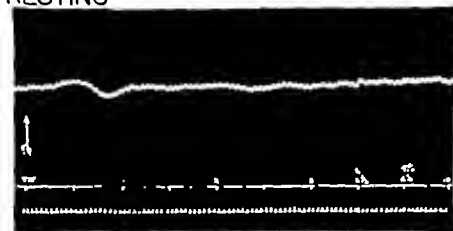


FIG 2. CAT 64—MECHOLYL (0.003 MG.) INJECTED INTO EACH JOINT

One joint rested and one exercised. Both had been injected with saline 13, 10, 6, and 3 days previously. Left hand tracings show greater immediate blood pressure effect with exercise. Right hand tracings show less residual mecholyl in washings from exercised joint.

CONTROL DATA

The method of experiment employed in these studies suggested the necessity of the following controls, in addition to those already cited.

The propriety of using distilled water as a vehicle for introducing mecholyl into joints was

checked by tests with two cats. A 0.003 mgm dose of mecholyl dissolved in distilled water was injected into one joint, and the same dose dissolved in cat serum, into the other joint. When both joints were kept at rest, there was no blood pressure effect in either case. Assay of the wash-

TABLE V*
Joints severely inflamed by injections with aleuronat

One joint injected with aleuronat both joints rested				One joint injected with aleuronat both joints exercised				Both joints injected with aleuronat one joint exercised						
Cat num ber	Greater blood pressure effect		Greater absorption		Cat num ber	Greater blood pressure effect		Greater absorption		Cat num ber	Greater blood pressure effect		Greater absorption	
	Normal joint	Ab- normal joint	Normal joint	Ab- normal joint		Normal joint	Ab- normal joint	Normal joint	Ab- normal joint		Rested joint	Exercised joint	Rested joint	Exercised joint
36		++		++++	48		++++		++++	58		++++		++++
44	?	?		++	49		++++		+	59		++++		++++
56		++++		++++	57		++++		++++	60		++++		++++
61	0	0	=	=										

* Mecholyl 0.003 mgm. was injected into each knee joint of each cat in each experiment.

The magnitude of observed difference in blood pressure effect, or in absorption, with respect to one of the two joints of a cat is indicated by the number of +s.

The symbol = indicates that the results were the same for the two joints.

The symbol 0 indicates that no effect occurred in this experiment.

constant although the plasma level was raised from 300 to 2400 mgm per cent. "It would appear that the glucose 'threshold' has its origin in the fact that the tubules can reabsorb glucose up to some constant, maximal quantity per unit time, if presented in excess of this quantity, the remainder is allowed to escape in the urine" (H. Smith.)

These observations give a very striking definition of the mechanism of glucosuria caused by intravenous infusions of glucose in the normal dog. The purpose of the present work is to determine whether the mechanism of glucose excretion in diabetic dogs is identical with that which has been found in the normal animal.

We have studied three dogs in which a diabetic condition had been induced by total pancreatectomy. The bladder was permanently fistulated by an ebonite cannula. These animals were kept in good condition for several months by daily injections of insulin. The total quantity of filtered glucose per minute may be computed by multiplying the creatinine clearance (filtration) by the concentration of glucose in the arterial plasma. The difference between this quantity and the amount of glucose excreted per minute gives the total amount of glucose reabsorbed by the tubules. Thus one may calculate the amount of glucose reabsorbed by the tubules from each cubic centimeter of glomerular filtrate, that is, from each cubic centimeter of a solution of glucose having the same concentration as the arterial plasma. We call this figure the "threshold," as, when expressed in grams per cent, it indicates which portion of the plasma glucose is not excreted under the conditions of the experiment.

METHODS

The experiments were performed without anesthesia, as they did not include any painful procedure. Two to 3 grams of creatinine dissolved in 200 cc. of tepid water were administered by stomach tube. Half an hour later, 500 cc. of water were given in the same way. When a marked diuresis was present, the urine was collected during a 2 to 5-minute period and at the same time a blood sample was drawn by femoral puncture. Glucose and creatinine were determined in the urine and in the arterial plasma.

The experiments on Dog I were all carried out when the animal had been fasting overnight, the purpose of this procedure being to keep the plasma glucose as constant as possible during the experiments. The blood

glucose of Dogs II and III was raised in most of the experiments by intravenous infusions of glucose-saline or by introducing a glucose solution into the stomach.

Blood. The blood was drawn by femoral puncture, collected in a tube containing some sodium fluoride and immediately centrifuged. A 1/10 dilution was made by adding to 2 cc. of plasma 16 cc. of distilled water, 1 cc. of 2/3 N sulfuric acid and 1 cc. of 10 per cent sodium tungstate solution, and filtering.

Urine. The method of West, Scharles, and Peterson (9) was used for the urinalyses, in order to avoid any decrease in reducing power. To 2 cc. of urine, 2 cc. of a 10 per cent solution of ferric sulfate were added, followed by 16 cc. of water. Two to 3 grams of powdered barium carbonate were then added to the solution. The mixture was thoroughly shaken and filtered. To the filtrate, a few milligrams of anhydrous sodium sulfate were added in order to precipitate the small amount of barium bicarbonate present. The precipitate was removed by centrifugation. This procedure yields urine dilutions absolutely colorless and without any turbidity.

Glucose. For Dog I, the colorimetric method of Benedict (10) was employed. Two cc. of tungstic filtrate of plasma and 2 cc. of diluted urine were used for the determinations. The urine, handled as described above, was diluted to such an extent that its final glucose concentration was nearly the same as that of the plasma filtrate. It was then possible to make the colorimetric readings for both filtrates against the color developed by the same glucose standard.

For Dogs II and III, the glucose analyses were made following the method of Shaffer and Somogyi (11). We allowed 2 cc. of filtrates of blood and urine to react with 2 cc. of copper solution number 50, containing 3 grams of KI per liter. The agreement between duplicates was very satisfactory.

Creatinine. Creatinine was determined by the method of Folin and Wu (12). The analyses were performed upon tungstic filtrates of plasma and upon urine dilutions adjusted to allow colorimetric readings against the standard used for the blood filtrate. A Burkner compensating colorimeter was used, supplied with a monochromatic filter. The readings were made with a stratum thickness of 20 mm and the 353 light filter. For the wavelengths transmitted by that light filter, the results conform with the Beer Lambert Law.

RESULTS

DOG I Female, 12 kgm

Pancreatectomy Dec 30, 1937 Vesical fistula
Jan 19, 1938

Ten experiments were done during a period of two months. The hyperglycemia being spontaneous, the range of plasma glucose extended only from 268 mgm per cent to 367 mgm per cent.

The results concerning this dog may be found in Table I and Figure 1

TABLE I
Data on Dog I

Experiment	Urine	Plasma glucose	Plasma creatinine	Glucose excreted	Creatinine excreted	Creatinine clearance (filtration)	Glucose excreted from 1 cc. of filtrate	Glucose reabsorbed from 1 cc. of filtrate
	cc. per min.	mgm. per cent.	mgm. per cent.	mgm. per minute	mgm. per minute	cc. per min.	mgm. per cent.	mgm. per cent.
I	3.40	268	7.75	6.90	5.03	65.0	10	258
II	2.50	274	6.50	8.25	3.36	53.2	15	259
III	4.00	280	10.40	21.70	5.92	57.0	38	242
IV	3.05	287	6.07	25.90	3.08	50.6	51	236
V	3.08	298	6.60	9.77	3.23	49.0	20	278
VI	3.70	310	6.01	28.20	4.91	70.0	40	270
VII	3.15	316	10.40	36.30	6.29	60.5	60	256
VIII	3.00	323	9.45	20.00	4.58	48.5	41	282
IX	2.39	346	11.00	23.70	4.73	43.0	55	291
X	3.10	357	9.10	30.00	4.64	51.4	58	299

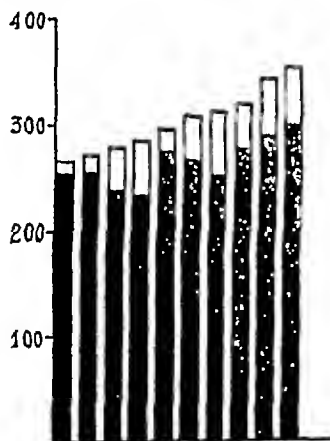


FIG. 1 DATA ON DOG I

Each column corresponds to a single experiment. Total height of the columns arterial plasma glucose (mgm. per cent). Black amount of glucose reabsorbed from each cc. of glomerular filtrate (mgm. per cent)

In these experiments, while the plasma glucose varied from 268 to 367 mgm. per cent, the quantity of glucose which was reabsorbed from 1 cc. of glomerular filtrate varied from 236 to 299 mgm. per cent. This indicates that in this range of moderate hyperglycemia, the amount of glu-

cose reabsorbed by the tubules increases when the blood sugar level rises

DOG II Female, 14 kgm

Pancreatectomy and vesical fistula Sept 14, 1937

Eight experiments were performed on this animal during a period of six weeks. The results may be found in Table II and Figure 2

TABLE II
Data on Dog II

Experiment	Urine	Plasma glucose	Plasma creatinine	Glucose excreted	Creatinine excreted	Creatinine clearance (filtration)	Glucose excreted from 1 cc. of filtrate	Glucose reabsorbed from 1 cc. of filtrate
	cc. per min.	mgm. per cent.	mgm. per cent.	mgm. per minute	mgm. per minute	cc. per min.	mgm. per cent.	mgm. per cent.
I	2.94	286	5.73	11.0	3.99	69.5	16	270
II	4.04	372	7.94	34.5	5.82	73.4	47	325
III	5.67	467	10.48	96.0	8.17	78.0	123	344
IV	3.30	482	8.82	91.5	6.46	73.2	125	357
V	3.23	514	9.70	75.0	5.91	61.0	123	391
VI	4.60	557	11.70	103.0	7.16	61.2	168	389
VII	5.58	570	6.54	138.0	4.86	74.4	185	385
VIII	5.91	610	13.98	135.0	8.65	61.8	218	392

Here, the blood sugar varied from 286 to 610 mgm. per cent. The amount of glucose which the tubules reabsorbed from one cc. of glomerular filtrate at first rises with increasing glycemia, but when the blood sugar exceeds 500 mgm. per cent the threshold remains practically constant (between 385 and 392 mgm. per cent)

DOG III Female, 13 kgm

Pancreatectomy and vesical fistula. Feb 22 1938

Twenty two experiments were performed during a period of four months. The blood sugar concentration was 325 to 950 mgm. per cent. In Experiments 1 to 4, the animals were fasting. In the other experiments the blood sugar was raised by an intravenous infusion of 100 to 200 cc. of 20 per cent glucose in saline. The collection of blood and urine was done from 2 to 5 minutes after the end of the infusion.

The results of these experiments may be found in Table III and Figure 3

The range of blood sugar extends from 325 mgm. to 957 mgm. per cent. The amount of glucose reabsorbed from 1 cc. of glomerular fil-

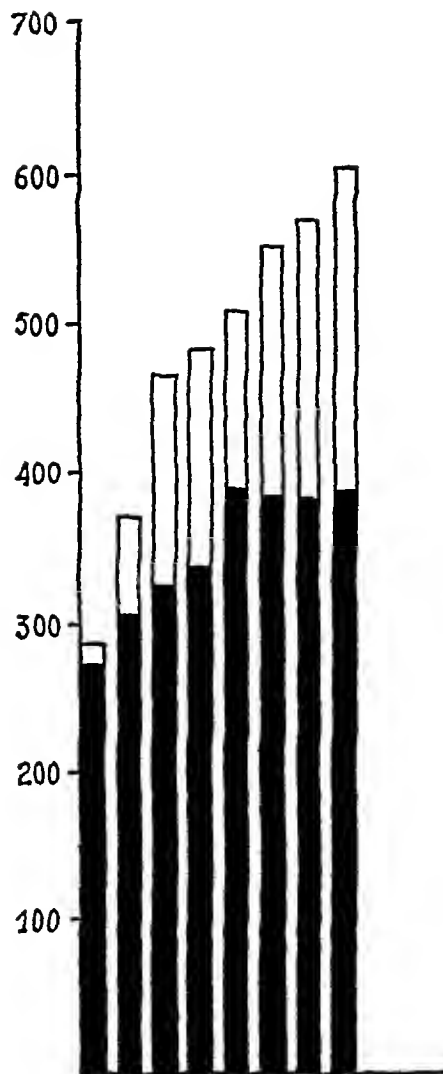


FIG 2. DATA ON DOG II

Each column corresponds to a single experiment. Total height of the columns arterial plasma glucose (mgm per cent) Black amount of glucose reabsorbed from each cc. of glomerular filtrate (mgm. per cent)

trate fluctuates between 287 and 394 mgm per cent, with a mean value of 347 mgm per cent

COMMENT

We may outline the results of our experiments in the following way When the concentration of glucose increases in the plasma, it rises to the same extent in the glomerular filtrate At a certain moment, the reabsorptive power of the tubular cells cannot keep pace with the amount of glucose which is presented, and some of the glucose escapes into the urine If the quantity presented

TABLE III
Data on Dog III

Experiment	Urine	Plasma glucose	Plasma creatinine	Glucose excreted	Creatinine excreted	Creatinine clearance (filtration)	Glucose excreted from 1 cc of filtrate	Glucose reabsorbed from 1 cc of filtrate
	cc per minute	mgm per cent	mgm per cent	mgm per minute	mgm per minute	cc per minute	mgm per cent	mgm per cent
I	4 30	325	4 94	29 4	4 59	93 0	32	293
II	0 45	349	17 80	13 9	13 23	74 3	19	330
III	3 25	352	5 33	33 8	4 85	91 0	37	315
IV	4 72	360	6 34	34 0	5 07	80 0	42	318
V	0 45	415	18 90	36 4	7 69	40 7	89	326
VI	2 93	421	15 20	63 0	13 77	90 4	70	351
VII	2 73	458	11 50	87 0	9 94	86 4	101	357
VIII	1 72	469	19 65	100 5	13 36	67 0	150	319
IX	2 50	521	22 10	133 0	13 32	60 4	220	301
X	5 67	524	23 00	134 0	13 00	56 5	237	287
XI	3 00	534	21 20	161 0	15 94	75 2	214	320
XII	5 50	535	22 00	183 0	18 53	84 2	217	318
XIII	2 75	537	6 13	128 0	5 23	85 4	150	387
XIV	2 20	551	13 20	140 0	8 93	67 6	207	344
XV	2 30	573	15 00	132 0	8 76	58 4	226	347
XVI	5 45	595	20 00	163 0	15 00	75 0	217	378
XVII	4 37	597	7 74	187 5	6 12	79 0	237	360
XVIII	5 20	603	8 12	167 8	6 46	79 5	211	392
XIX	2 78	627	16 00	167 5	11 52	72 0	233	394
XX	5 35	778	7 12	333 0	5 25	73 6	452	326
XXI	5 85	828	8 45	360 0	5 85	69 2	520	308
XXII	7 40	957	9 72	410 0	6 49	65 8	623	334

increases further, the reabsorption increases also, but nevertheless it is more and more overburdened If the plasma glucose continues rising, the reabsorptive capacity of the tubular cells becomes completely saturated, and the quantity of reabsorbed glucose remains practically constant at this maximal value for any further increase of glycemia It is very tempting to compare the renal function under those circumstances with that of the phlorizinized dog In the latter, the reabsorption of glucose is completely blocked by phlorizin and consequently the total amount of filtered glucose is found in the bladder urine In the diabetic dog, a constant part of the filtered glucose saturates the reabsorptive power of the tubular cells and the remainder is completely excreted as it should be in a phlorizinized dog The blocking of reabsorption which in the phlorizinized dog is produced by a drug is caused in the diabetic animal by the glucose itself

These conclusions are based on the mean values of our results When comparing single experiments, one finds that the "threshold" fluctuates appreciably The meaning of these fluctuations

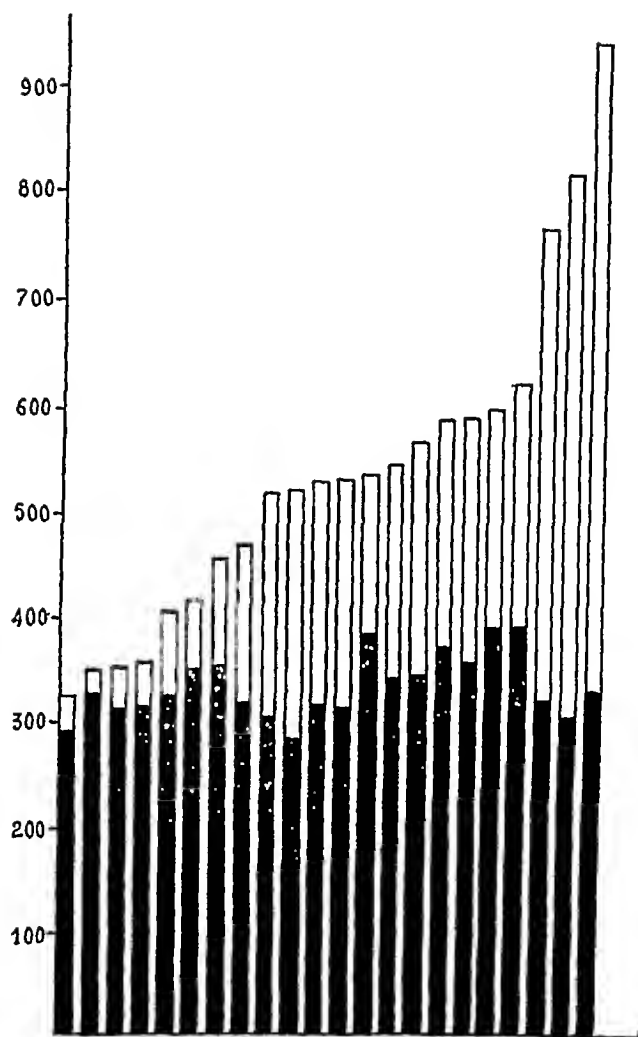


FIG. 3. DATA ON DOG III

Each column corresponds to a single experiment. Total height of the columns arterial plasma glucose (mgm. per cent) Black amount of glucose reabsorbed from each cc. of glomerular filtrate (mgm. per cent)

is difficult to interpret for the moment. They may be owing in great part to the unavoidable and cumulative errors in collecting urine and blood, and in the chemical determinations. Furthermore, it is very likely that the reabsorptive power of the tubular cells may be influenced by several factors, for instance variations in the number of functioning glomeruli or modifications in the volume of fluid filtered by single glomeruli, changing the rate of flow through the tubules. Other factors may be of importance, such as hormonal influences, but they are still to be determined.

Our observations prove that the mechanism of glucose elimination in diabetic dogs is the same as that found by Fisher and Shannon in normal dogs when a high level of glycemia was maintained by a continuous injection of glucose-saline. It is worth noticing that the renal threshold in our diabetic dogs did not undergo any appreciable change during a period of observation which lasted several months. Moreover, the threshold, defined by the maximal quantity of glucose reabsorbed by the tubular cells from 1 cc. of glomerular filtrate is not the same for each dog, as it is about 390 mgm per cent for Dog II and 347 mgm per cent for Dog III.

The threshold is variable in the range of moderate hyperglycemia, it rises when the blood sugar increases. When the level of hyperglycemia is high, the threshold stays at a maximal value which is not the same in different individuals. These facts explain why the renal threshold presents itself both as a permanent individual character and as a rather elusive value, varying appreciably when the level of blood sugar is only moderately raised.

SUMMARY

1 In dogs made diabetic by pancreatectomy, the excretion of glucose and creatinine has been studied.

2 Considering the creatinine clearance as a measure of the glomerular filtration and the glucose concentration of the arterial plasma as equal to the concentration of this substance in the glomerular filtrate, one may calculate the quantity of glucose which the tubular cells reabsorb from 1 cc. of glomerular filtrate. This figure is called

the "threshold." When the blood sugar concentration increases moderately, this threshold increases. With further elevation of the blood sugar, the threshold soon reaches a maximal value which remains constant for any higher level of glycemia.

3 In the diabetic dog, at high levels of hyperglycemia, a constant part of the filtered glucose saturates the reabsorptive power of the tubular cells and the remainder is completely excreted as in a phlorizinized dog.

4 These characteristics of the renal function did not change appreciably in the diabetic dogs during a period of observation which lasted several months.

BIBLIOGRAPHY

- 1 Richards, A. N., *Methods and Results of Direct Investigations of the Function of the Kidney*. Williams and Wilkins Co., Baltimore, 1929.
- 2 Richards, A. N., and Walker, A. M., *Urine formation in the amphibian kidney*. *Am J M Sc*, 1935, 190, 727.
- 3 Rehberg, P. B., *Studies on kidney function. The rate of filtration and reabsorption in the human kidney*. *Biochem J*, 1926, 20, 447.
- 4 Marshall, E. K., Jr., *The comparative physiology of the kidney in relation to theories of renal secretion*. *Physiol Rev*, 1934, 14, 133.
- 5 Smith, H. W., *The Physiology of the Kidney*. Oxford University Press, New York, 1937.
- 6 Poulsson, L. T., *On the mechanism of sugar elimination in phlorrhizin glycosuria*. *J Physiol*, 1930, 69, 411.
- 7 Jolliffe, N., Shannon, J. A., and Smith, H. W., *The excretion of urine in the dog. III. The use of nonmetabolized sugars in the measurement of the glomerular filtrate*. *Am. J Physiol*, 1932, 100, 301.
- 8 White, H. L., and Monaghan B., *A comparison of the clearances of various urinary constituents*. *Am. J Physiol*, 1933, 104, 412.
- 9 Govaerts, P., and Cambier P., *Elimination comparée du glucose, de la créatinine et de l'urée sous l'influence de la phlorizine*. *Bull. Acad roy de-med. de Belgique*, 1934, 14, 226.
- 10 West, E. S., Scharles, F. H., and Peterson, V. L., *The determination of true sugar in blood*. *J Biol Chem.*, 1929, 82, 137.
- 11 Benedict, S. R., *Determination of sugar in blood*. *J Biol Chem.*, 1929, 83, 165.
- 12 Shaffer, P. A., and Somogyi, M., *Copper iodometric reagents for sugar determinations*. *J Biol Chem.*, 1933, 100, 695.
- 13 Folin, O., and Wu, H., *A system of blood analysis*. *J Biol Chem.*, 1919, 38, 81.

A DIRECT METHOD FOR THE DETERMINATION OF VENOUS PRESSURE, RELATIONSHIP OF TISSUE PRESSURE TO VENOUS PRESSURE

By GEORGE E. BURCH AND WILLIAM A. SODEMAN

(From the Department of Medicine Tulane University of Louisiana School of Medicine and the Charity Hospital of Louisiana New Orleans)

(Received for publication August 22, 1938)

Previous observations (2) indicate that there is a positive pressure in the tissue spaces. It is obvious that any pressure manipulations of the tissues must take into consideration this extravascular, or tissue, pressure. Tissue pressure is exerted upon all the contained structures including the blood vessels and opposes the intravascular pressure. In the arterial tree, where the intravascular pressures are high, this is relatively insignificant. However, in the venous vessels, where the intravascular pressure approaches the tissue pressure, particularly above heart level, this effect becomes increasingly important. The present study is concerned with an evaluation of the interplay of these pressures and their influence upon the determination of venous pressure by indirect methods.

METHODS AND MATERIALS

The indirect measurements of venous pressure were made according to the method of Hooker (1). Definite collapse of the vessel was used as the end point. Collapse was never carried beyond the level of the adjacent skin. Tissue pressure determinations were made by the technic previously described (2). The direct determinations of venous pressure were made with the apparatus employed in the tissue pressure measurements. However the technic was modified as follows: A 2 per cent sodium citrate solution was used instead of normal sodium chloride. The solution was drawn about half way up the adapter to compensate for capillarity as in tissue pressure determinations and the pressure within the system brought to atmospheric. Upon insertion of the needle into the vein the manometer in the adapter slowly began to rise. The pressure in the system was gradually increased until the movement of the manometer ceased and there was no movement into or out of the vein. This was taken as the venous pressure.

Studies were made on ten normal subjects. Only subjects with prominent veins in the dorsum of the hand were chosen so as to facilitate the reading of the end point in the indirect venous pressure method. Subjects rested in the sitting position with the hand at heart level fourth intercostal space. Indirect and direct venous pres-

ures were determined for the same portion of the same vein on the dorsum of the hand. The subcutaneous tissue pressure was then measured in the tissues immediately surrounding the same portion of the vein.

Determinations were made in the order given. The observer who read the end points was ignorant of the water manometer readings, which were recorded by another individual. In this way subjective influences were eliminated. Three readings were made in each instance.

RESULTS

The results are given in Table I. Invariably, the indirect determinations were lower than the

TABLE I
Venous pressure and tissue pressure values in cm. of water at heart level in ten normal subjects

Subject number	Venous pressure direct	Venous pressure indirect	Tissue pressure	Indirect venous pressure + tissue pressure	Difference between direct venous pressure and indirect venous pressure + tissue pressure
	cm. H ₂ O	cm. H ₂ O	cm. H ₂ O	cm. H ₂ O	cm. H ₂ O
1	14.2	11.0	1.8	12.8	-1.4
2	14.6	9.4	5.4	14.8	+0.8
3	13.0	9.2	2.6	11.8	-1.2
4	7.8	6.0	3.2	9.2	+1.4
5	13.2	7.4	2.9	10.3	-2.9
6	12.4	11.8	2.2	14.0	+1.6
7	7.8	5.2	3.2	8.4	+0.6
8	9.6	8.4	2.6	11.0	+1.4
9	9.4	7.6	1.8	9.4	0.0
10	10.4	8.4	3.0	11.4	-1.0
Mean	11.2	8.4	2.9	11.3	+0.1

corresponding direct determinations. The average indirect value was 8.4 cm. of water and the average direct value was 11.2 cm. of water. The average determination of tissue pressure was 2.9 cm. of water. Individual determinations may be seen in Table I. It is also evident in Table I that the addition of the indirect venous pressure and tissue pressure closely approximates the direct venous pressure the mean difference being only 0.1 cm. of water.

increasingly important as the venous pressure decreases

BIBLIOGRAPHY

- 1 Hooker, D R, The influence of age upon the venous blood pressure in man. *Am. J. Physiol.*, 1916, 40, 43
2. Burch, G E. and Sodeman, W A, The estimation of the subcutaneous tissue pressure by a direct method *J Clin. Invest.*, 1937, 16, 845
- 3 Landis, E. M, Micro-injection studies of capillary blood pressure in human skin *Heart*, 1929-31, 15, 209
- 4 Eyster, J A E., *The Clinical Aspects of Venous Pressure.* Macmillan Company, New York, 1929, p 30
- 5 Brams, W A., Katz, L N and Schutz, W J, Intravenous pressure. I New method of determination *Arch. Int. Med.*, 1933, 51, 33
- 6 McIntire, J M and Turner, A H, Venous pressure and posture in normal young women *J Clin. Invest.*, 1935, 14, 16
- 7 Hooker, D R., Observations on the venous blood pressure in man *Am. J. Physiol.*, 1914, 35, 73
- 8 Krogh, A, Turner, A H and Landis, E. M, A celluloid capsule for measuring venous pressure. *J Clin. Invest.*, 1932, 11, 357

STUDIES IN SERUM ELECTROLYTES XII THE EFFECT OF WATER RESTRICTION IN A PATIENT WITH ADDISON'S DISEASE RECEIVING SODIUM CHLORIDE¹

By DONALD M. WILLSON AND F. WILLIAM SUNDERMAN

(From the William Pepper Laboratory of Clinical Medicine University of Pennsylvania Philadelphia)

(Received for publication August 18, 1938)

The efficacy of sodium salts in the treatment of adrenal cortical insufficiency in man and experimental animals has been recognized, and the changes in the distribution and excretion of water and electrolytes following their therapeutic use have been investigated (1, 2, 3, 4). This present study is concerned with the effect of variations in the total water consumption of a patient with Addison's disease receiving a relatively constant high intake of sodium chloride.

Observations in the literature concerning adrenal insufficiency suggest that the changes in the distribution of body water are secondary to changes in the distribution of sodium and potassium. The forced ingestion of water by mouth is apparently ineffective *per se* in preventing the development of hemoconcentration observed in insufficiency. If sufficient water be given an adrenalectomized dog receiving no cortical extract and on low intake of salt, a fairly constant fluid balance may be obtained and yet hemoconcentration may develop (5).

Hemoconcentration and dehydration are commonly observed in severe crisis. The hemoconcentration is recognized by the increased concentration of hemoglobin, the increased percentage of erythrocytes, and the small proportion of serum yielded from blood. Studies in experimental insufficiency indicate that the blood volume is diminished (6) and that the amount of interstitial fluid may be reduced as well (7). The diuresis that frequently occurs with the onset of a crisis is apparently inadequate to account for the diminution in serum volume (8). Harrop suggests that the most important factor responsible for the observed hemoconcentration is a "movement of extracellular fluid into the tissue cells" (7).

Upon withdrawal of cortin from adrenalectomized dogs maintained on a sodium and chloride free diet, Swingle, Parkins, Taylor, and Hays (9) observed that hemoconcentration and circulatory collapse occurred without the loss of sodium, chloride, or water by way of the urine. Furthermore, the injection of massive doses of cortin alone brought about a dilution of the blood and a relief of symptoms. Although not entirely apparent from their data they concluded that the injection of cortical extract mobilized the accumulation of intracellular fluid and electrolytes, and shifted them from the intracellular to the extracellular and vascular compartments.

In an effort to obtain further information upon the problems of hemoconcentration and the internal shift of water in adrenal insufficiency, this study of water and electrolyte metabolism in Addison's disease was undertaken.

PROCEDURE

A patient with typical Addison's disease who had remained reasonably well-controlled on a normal diet with added salt for several months (see protocol) was selected for these observations. The experimental procedure consisted of a continuation of this dietary regimen, but with variations in the intake of water to produce periods of oliguria and polyuria.

One of us (D.M.W.) was used as a control subject for these observations under identical conditions of diet and intake of salt and water as were imposed upon the patient, but without the same limitations of activity.

A special metabolic ward and nursing staff were utilized to facilitate this investigation. Two standard diets were hashed and analyzed for their content of chloride and water. Duplicates of these two rations were served on alternate days throughout the study.

The total intake of water was calculated as the sum of the intrinsic water of the diet, beverage water and water of oxidation of the food constituents. Intrinsic water was determined by the difference between the wet and dry weights of the two complete sample diets. The water of oxidation was computed as 1.07 grams per gram fat, 0.555 gram per gram carbohydrate, and 0.413

¹ Aided by a grant from the Faculty Research Committee, University of Pennsylvania, Philadelphia, Pennsylvania.

On the tenth experimental day a relatively large volume of urine was excreted. The concentrations of sodium and chloride remained elevated in spite of this increased volume and the total amounts of sodium and chloride excreted attained the range observed during the days of the previous control period. Hence, during the dehydration period sodium and chloride were apparently retained by the body awaiting only adequate diuresis for its excretion.

The loss of four and a half pounds of body weight during this seven-day period attests the rigidity of fluid restriction. As would be expected in one whose liquids had been so stringently reduced, the patient soon became exceedingly thirsty. Mouth, lips, and throat were very dry and uncomfortable. Cheeks and eyes became sunken, pigmentation was more pronounced, and it became difficult for her to eat all of the daily ration because of dryness. Although anorexia and weakness increased, it was not until the last two days of dehydration that they became seriously aggravated. During these final days the patient was too weak to get out of bed, and lay quietly all day, moistening her lips with ice. Tissue turgor was markedly diminished, but no change in blood pressure was noted. In spite of the rigors of this regimen, the patient professed willingness to continue and was conscientious in expectorating all of the melted ice but was unable to take her full quota of salt during the final twenty-four-hour period.

Dehydration in the control subject

The effects of dehydration in the control subject are interpolated at this point for comparison with the results obtained from the observations on the patient, and the data are shown in Table II and Figure 2B. Under identical conditions (excepting that activity was not restricted) the volumes of urine in the control subject diminished to levels comparable with those of the patient, and in the course of six days, there was a loss of five pounds in body weight. As dehydration developed, the concentration of sodium and of chloride in the urine gradually increased until on the final day it was about 60 per cent above the maximum concentration exhibited in the urine of the patient, and the continuing rise in the con-

centration curve (Figure 2B) suggests that the limits of the concentrating power might not have been reached. No retention of sodium or chloride occurred during this phase of water deprivation.

Post-dehydration period in patient with Addison's disease

Upon the removal of fluid restriction our patient consumed three liters of water within eight hours, and a rapid improvement in general condition was noted, the extreme anorexia disappeared and moderate physical activity was quickly resumed. The concentration of hemoglobin dropped 28 grams per 100 cc in twenty-four hours, suggesting rapid dilution of the blood. It required several days, however, for the body weight, deepened pigmentation, and diminished tissue turgor to return to their usual state. The great increase of ingested water the first day following dehydration was not reflected by appreciable diuresis and concentrations of sodium and chloride in the urine on this day reached maximum levels (Table I). On subsequent days the volume of urine increased, and rapid fall in concentrations of sodium and chloride was noted. This occurred in spite of the previous retention of sodium and chloride. No diarrhea was present which might have carried off the retained salt.

Effects of a high fluid intake on patient with Addison's disease (Water forced)

The next step in the investigation consisted of an attempt to cause an acceleration of the loss of sodium and chloride from the body through the urine by means of a diuresis induced by a high intake of fluid. Over an eight-day period, ingested water approximated 4,500 cc per day resulting in the excretion of large volumes of urine. The concentration of sodium and chloride in the urine decreased, but the average daily output of these ions was greater than for any period excepting the preliminary control period.

The total daily urinary excretion of sodium and chloride in the control subject following diuresis by the ingestion of water was essentially the same as obtained during the period of dehydration. Apparently, within the scope of this experiment, the normal kidney easily excretes relatively large

amounts of salt whether fluids are restricted or forced

Final control period

The patient was followed for an additional six days during which time fluids were ingested as desired. No unusual change in the excretion of sodium and chloride occurred during this period of observation.

Chloride and sodium balances according to periods

A summation of the uncorrected chloride balances (Table IIIA) shows agreement in the third and fifth periods (control) but relative to these, a retention in the first period. From this it is inferred that at the start of these observations the patient might not have been quite in balance and that a consideration of the data according to periods might better begin with the second (de-

hydration) period. This has been undertaken in Tables IIIB and IIIC. In Table IIIA it will be observed that the ratio of sodium to chloride excretion in the urine is fairly constant throughout all of the periods of study.

In Table IIIB are presented our calculations of the corrected daily chloride balance for the last four periods. For the purpose of these calculations it is assumed that a complete chloride balance was achieved at the end of the fifth period and that throughout the total period of experimentation there was a constant daily loss of chloride amounting to 44.8 m.eq. from other channels than urine.⁴ In Table IIIC are presented our evaluations of the sodium output assuming that its intake is proportional to the chloride intake and that there occurred a constant loss of

$$\frac{45765 \text{ m.eq.} - 4596 \text{ m.eq.}}{26 \text{ days}} = 44.8 \text{ m.eq. per day}$$

TABLE III
(A)
Summary of data on patient with adrenal insufficiency

Period	Days	Cl intake	Cl output in urine	Cl intake less Cl output in urine	Uncorrected average daily Cl balance	Na output in urine	Na output in urine Cl output in urine
		m. eq.	m. eq.	m. eq.	m. eq.	m. eq.	ratio
1 Control	6	1378	1242	136	+ 23	1169	0.94
2 Dehydration	7	1469	1002	467	+ 67	894	0.89
3 Water as desired—control	5	1157	906	251	+ 50	850	0.94
4 Water forced	8	1750	1610	140	+ 17	1410	0.88
5 Control	6	1389	1078	311	+ 52	997	0.93

(B)
Cumulative chloride balance

Period	Total days	Cumulative Cl intake	Cumulative Cl output in urine	Cumulative corrected Cl output	Cumulative Cl balance	Cl balance for period	Corrected average daily Cl balance
		m. eq.	m. eq.	m. eq.	m. eq.	m. eq.	m. eq.
2 Dehydration	7	1469	1002	1316	+153	+153	+21.9
3 Water as desired	12	2626	1908	2448	+178	+ 25	+ 5.0
4 Water forced	20	4376	3518	4418	- 42	-220	-27.5
5 Control	26	5765	4596	5765	0	+ 42	+ 7.0

(C)
Cumulative sodium balance

Period	Total days	Cumulative Na intake	Cumulative Na output in urine	Cumulative corrected Na output	Cumulative Na balance	Na balance for period	Corrected average daily Na balance
		m. eq.	m. eq.	m. eq.	m. eq.	m. eq.	m. eq.
2 Dehydration	7	1469	894	1329	+140	+140	+20.0
3 Water as desired	12	2626	1744	2489	+137	- 3	- 0.6
4 Water forced	20	4376	3154	4396	- 20	-157	-19.6
5 Control	26	5765	4151	5765	0	+ 20	+ 3.3

62.1 m eq of sodium per day from other channels than urine³

Allowing for uncertainties in these assumptions the picture would seem to be fairly clear—there was retention of both sodium and chloride during the dehydration period and a washing out of these ions in the forced water period

Serum analyses

The results of the analyses of the serum taken during various phases of this experimental procedure are given in Table IV. It will be seen

TABLE IV
Serum studies in patient with adrenal insufficiency

Period	Body weight	Sodium	Chloride	Sodium content	Chloride content	Serum volume
	kilos	m eq per liter	m eq per liter	m eq	m eq	ml per kgm body weight
Preliminary control	60.5	129.4	100.7			
Dehydration	58.4	140.3	107.1	268.6	205.6	32.8
Water forced	59.4	127.5	103.0	286.8	231.6	37.9
Final control	59.1	126.8	101.6	285.0	228.3	38.0

that the concentration of sodium in the serum was low in the preliminary control period, forced water, and final control periods. In these same periods the chloride concentrations were within the normal range. During the period of dehydration both the sodium and chloride concentrations in the serum were increased to the upper normal range for these components or even higher for sodium.

Studies of serum volume in this patient were of particular interest. During the period of dehydration the serum volume for this patient was 32.8 ml per kgm of body weight, which is the lowest value for serum volume obtained in this laboratory. Moreover, the serum at this time yielded evidence of an increased concentration of the serum proteins since the specific gravity was 1.0295 at 20°/20°. The volumes of serum per kilogram of body weight measured during the final control period and during the phase of forced fluid intake were 38.0 and 37.9 ml respectively.

$$\frac{5765 \text{ m.eq} - 4151 \text{ m.eq}}{26 \text{ days}} = 62.1 \text{ m.eq per day}$$

These values are still considerably below our range of values for normal individuals (45 to 55 ml).

The control subject differed from the patient with Addison's disease in that variations of fluid intake had no effect upon the concentration of the electrolytes in the blood serum. Likewise, the serum volume in the control subject was essentially the same during dehydration and hydration.

DISCUSSION

Impairment of renal function in our patient is suggested by the retention of sodium and chloride during the period of oliguria, in contrast to the lack of such retention in the normal individual studied under the same conditions. In addition, the patient had a diminished urea clearance varying from 23 to 33 per cent of average normal function. It would appear noteworthy that a similar diminution occurs in adrenalectomized dogs following withdrawal of extract (14). We believe these changes must be the result of the failure of the adrenals alone, since the presence of coincidental renal disease was excluded by the absence of antecedent history, and the failure to find casts, albumin, etc., in any of the numerous specimens of urine examined.

SUMMARY

Studies are reported of the effects of water restriction and of the forced water ingestion, respectively, in a patient suffering with chronic adrenal cortical insufficiency who, throughout the period of observation, had received a high daily intake of sodium chloride. For several months previous to our studies the patient's condition had been adequately controlled by a similar high daily intake of sodium chloride and by the ingestion of fluids as desired.

During the period in which water was restricted our studies indicated a diminished ability of the patient to concentrate sodium and chloride in the urine, and a significant retention of these ions by the individual. During this same period there was a marked elevation in the concentrations of sodium and chloride in the blood serum but, since the increase in concentration of these components was associated with a shrinkage of the serum

volume, the total quantities of sodium and chloride in the circulating serum were actually reduced.

A normal individual studied under similar conditions excreted sodium and chloride in concentrations approximately 60 per cent greater than the patient and no retention of these ions occurred. In the normal individual, measurements of serum volume were essentially the same during the periods of fluid restriction and forced fluid ingestion.

Under the conditions of these observations the simple restriction of water resulted in the development of symptoms of severe adrenal insufficiency. Resumption of a normal intake of water resulted in a return of the patient to her normal state of health.

The forced ingestion of water in this patient had no appreciable influence upon the serum volume or the concentrations of sodium and chloride in the serum.

PROTOCOL

N P (Hospital Number 35-19589) a 54-year-old Italian-born white woman the sole support of her family had worked at a loom in a woolen mill for the previous twenty years. At the age of thirty two she suffered from pleurisy and an abdominal enlargement relieved by paracentesis. Progressive weakness, anorexia, and pigmentation were first noted in the fall of 1934 and she was advised to take sodium chloride. The symptoms increased, however, and a year later, because of extreme weakness occasional vomiting, marked pigmentation and a loss of 46 per cent of body weight, the patient was admitted to the University Hospital.

Upon examination she appeared thin, tired, and weak, with dry, inelastic skin hanging loosely in folds. A diffuse, muddy brown pigmentation was present, with accentuation upon the lips, neck, distal portions of the extremities, and several scars. It was also present at the lingual borders and upon the buccal mucosa at points of contact with the teeth. Blood pressure was 108 mm.Hg systolic and 78 diastolic. The right half of the thoracic cage was flattened, and expanded poorly with respiration. Resonance in this area was impaired and the transmission of voice and breath sound was poor. There was also a right ovarian cyst.

Laboratory data on admission were: Erythrocytes 4,400,000, hemoglobin 13.3 grams per 100 ml., leukocytes 8,100 with a normal differential distribution. The cell volume was 49 per cent with a cell volume index of 1.23. Urine analysis revealed no abnormalities. The concentration of the urea nitrogen of the blood was 18 mgm. per

100 ml. In the serum the concentration of chlorides was 98.3 meq per liter, total base, 135.9 meq per liter, and cholesterol 177 mgm. per 100 ml.

The patient was placed on a high intake of sodium chloride, and for six weeks showed a gradual improvement. During this period, the systolic blood pressure ranged between 110 and 92 mm.Hg and the diastolic ranged between 72 and 64. Abdominal pain in upper left quadrant was occasionally present, and three bloody mucus streaked stools were noted shortly before her discharge from the hospital.

Blood counts during the period of hospitalization remained practically unchanged and urine analyses were essentially normal although the range of specific gravities was low (1.005 to 1.012). Serological reactions were negative, and a normal glucose tolerance curve was obtained. Roentgenograms disclosed a thickening of the entire pleura on the right side, with calcification at the base, and multiple areas of calcification in the upper abdomen, which were not limited to adrenal areas.

The patient was readmitted in July 1935 because of an exacerbation of symptoms. Weakness had increased, vomiting frequently occurred and abdominal pain in upper left quadrant was again distressing. In addition, there had developed multiple joint symptoms consisting of stiffness and a dull aching pain, aggravated by motion. The erythrocytes were now 5,400,000 and the hemoglobin was 15.9 grams per 100 ml. The urea nitrogen of the blood was 16 mgm. per 100 ml., and in the serum the chlorides were 99.8 meq per liter, CO_2 44 volumes per cent and cholesterol, 273 mgm. per 100 ml. Although the systolic blood pressure was 112 mm.Hg the evidences of hemoconcentration, the episodes of vomiting and the general appearance of the patient gave the impression that she was in a state of mild crisis.

Therapy consisting of 18 grams of NaCl and 2.5 cc. of anterior pituitary extract daily was begun, and in five days there was considerable improvement. Vomiting subsided, anorexia diminished and the patient became ambulatory. Concurrently the erythrocytes fell to 4,400,000, the hemoglobin to 13.7 grams per 100 ml., the urea nitrogen to 9.5 mgm. per 100 ml. In the serum, the chlorides rose to 105.2 meq per liter and the sodium was found to be 125.0 meq per liter. Continuation of this therapy for an additional three weeks resulted in no further improvement, the concentration of sodium in the serum remaining well below normal (123.9 meq per liter) nor did weekly injections of 5 cc. of eschatin appear to have any added effect.

Therapy was then limited to the daily use of 6 grams of NaHCO_3 and 9 grams of NaCl. The concentration of sodium in the serum following this treatment was 127.8 meq per liter. Although some anorexia, weakness, and discomfort in the various joints persisted, there was great clinical improvement and the patient was discharged six weeks later with instructions to continue this simple therapy.

She remained quite well until the development of an

upper respiratory infection. Following this, anorexia and weakness increased, and the generalized arthritic pains became quite troublesome, causing chief concern in the mornings, and diminishing in severity as the day progressed. Although weakness and languor were a little more apparent, she did not seem to be in crisis. Results of analyses of blood and serum showed little variation from those of the previous discharge, with the exception of an elevation in urea nitrogen to 30 mgm. per 100 ml. of blood. Upon active resumption of NaCl and NaHCO₃ therapy, an immediate improvement was noted.

Therapeutic results in this patient, while beneficial, had left much to be desired, and an effort was made to determine the effectiveness of one of the newer adrenal cortical extracts. The total intake of sodium chloride was lowered to 4 grams daily for seven days. On the fourth day the expected aggravation of symptoms was noted, and daily intramuscular injections of the extract were begun. In spite of this treatment a gradual progression into crisis occurred, which was relieved by a return to that form of therapy which previously had been most beneficial (9 grams NaCl and 6 grams NaHCO₃ daily). Analysis of the serum confirmed the clinical impression that the cortical hormone, as used in this patient, was ineffective in preventing the development of crisis under conditions of salt restriction.

Date	Na	Cl	Blood urea nitrogen	Remarks
	m. eq per liter	m. eq per liter	mgm per 100 ml.	
Dec. 18				Low salt intake begun. Increased weakness and anorexia. Daily 5 cc. adrenal cortical extract* in addition to low salt intake.
Dec. 19	126.0	99.2	32	
Dec. 25	119.6	94.1	34	Extreme weakness and vomiting. I. v. saline, high NaHCO ₃ and NaCl begun.
Dec. 28	133.7	105.3	18	Much improved.

* Extract supplied by Upjohn and Co., Kalamazoo, Michigan.

Following this observation, the patient's clinical condition remained stationary under treatment consisting of daily administrations of 9 grams NaCl and 6 grams NaHCO₃, although some weakness and varying degrees of anorexia persisted. An attempt to improve this status with the use of cortical extract in addition to the sodium therapy resulted in no apparent change in general appearance or subjective state. Studies of the serum were unsatisfactory in the evaluation of the effect of these injections since essentially normal electrolyte levels had been maintained by means of the sodium salts alone and further increases were not expected from specific treatment.

During the eight-week period which covered the studies reported in the text the patient received only sodium chloride. On this regimen the patient's general condition was not quite so satisfactory as when bicarbonate was

also administered in conjunction with NaCl. Periodically, there was vomiting and mild exacerbations of weakness. Whether this reflected merely the omission of bicarbonate or was due to the relatively high amount of potassium included in the special diet is problematical.

With the conclusion of the results reported, the patient desired to leave the hospital for a short time before attempting any further studies. Accordingly, she returned to her home in another city with instructions to take the sodium chloride and bicarbonate that had been most effective in relieving her symptoms. A sudden illness of two days' duration however, resulted in her death five weeks later. No autopsy was performed.

BIBLIOGRAPHY

1. Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J., Electrolyte balance studies in adrenalectomized dogs with particular reference to the excretion of sodium. *J. Exper. Med.*, 1933, 57, 775.
2. Harrop, G. A., Soffer, L. J., Ellsworth, R., and Trescher, J. H., Studies on the suprarenal cortex. III. Plasma electrolytes and electrolyte excretion during suprarenal insufficiency in the dog. *J. Exper. Med.*, 1933, 58, 17.
3. Swingle, W. W., Pfiffner, J. J., Vars, H. M., and Parkins, W. M., The effect of fluid deprivation and fluid intake upon the revival of dogs from adrenal insufficiency. *Am. J. Physiol.*, 1934, 108, 144.
4. Harrop, G. A., Soffer, L. J., Nicholson, W. M., and Strauss, M., Studies on the suprarenal cortex. IV. The effect of sodium salts in sustaining the suprarenalectomized dog. *J. Exper. Med.*, 1935, 61, 839.
5. Harrop, G. A., Nicholson, W. M., and Strauss, Margaret, Studies on the suprarenal cortex. V. The influence of the cortical hormone upon the excretion of water and electrolytes in the suprarenalectomized dog. *J. Exper. Med.*, 1936, 64, 233.
6. Swingle, W. W., Vars, H. M., and Parkins, W. M., A study of the blood volume of adrenalectomized dogs. *Am. J. Physiol.*, 1934, 109, 488.
7. Harrop, G. A., The influence of the adrenal cortex upon the distribution of body water. *Bull. Johns Hopkins Hosp.*, 1936, 59, 11.
8. Swingle, W. W., Pfiffner, J. J., Vars, H. M., and Parkins, W. M., The relation between blood pressure, blood urea nitrogen, and fluid balance of the adrenalectomized dog. *Am. J. Physiol.*, 1934, 108, 428.
9. Swingle, W. W., Parkins, W. M., Taylor, A. R., and Hays, H. W., The influence of adrenal cortical hormone upon electrolyte and fluid distribution in adrenalectomized dogs maintained on a sodium and chloride free diet. *Am. J. Physiol.*, 1937, 119, 684.
10. Magnus-Levy, C. von Noorden, *Metabolism and Prac-*

- tical Medicine. W D Keener Chicago, 1907, p. 392.
- 11 Sherman H. C., The Chemistry of Food and Nutrition. Macmillan Co., New York, 1935, 5th ed., Table 62, pp 590-594
- 12 Wilder R. M., Kendall E. C., Snell A. M., Kepler E. J., Ryncarson, E. H., and Adams Mildred, Intake of potassium, an important consideration in Addison's disease. Arch. Int. Med., 1937, 59 367
- 13 Sunderman, F W., and Austun, J H., The measurement of serum volume. Am. J. Physiol., 1936, 117, 474
- 14 Stahl, J., Atchley D W., and Loeb, R. E., Observations on adrenal insufficiency J Clin. Invest., 1936 15 41
- 15 Sunderman, F William, Studies of serum electrolytes VII. The total base and protein components of the serum during lobar pneumonia with a note on the gastric secretion. J Clin. Invest., 1931, 11, 615

A LONG TERM STUDY OF THE VARIATION OF SERUM CHOLESTEROL IN MAN¹

By KENNETH B. TURNER AND ALFRED STEINER

(From the Research Division of Chronic Diseases Department of Hospitals City of New York and the Department of Medicine College of Physicians and Surgeons Columbia University, and the Presbyterian Hospital New York City)

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Although there are innumerable reports on the blood cholesterol of man under normal or abnormal conditions, most of these are based on single or, at best, a few determinations on a given individual. While there has developed an increasing realization that the variation or the lack of change in the level of blood cholesterol of the individual under a certain set of conditions is of paramount importance, reports of such variations are meager.

An unusual opportunity was afforded us to study the serum cholesterol of a group of hospitalized but not acutely ill patients at approximately weekly intervals for a year or more.

MATERIAL AND PLAN OF STUDY

There were 10 patients in the group. The following 9 of these were studied for 12 to 14 months: D. C., a Negro of 21 years with inactive rheumatic heart disease; M. C., a white male, age 62, with hypertensive vascular disease; M. C., a white female, age 55 with diabetes; H. F., a white female, age 42, with tabes dorsalis; F. K., a white male, age 64 with diabetes; J. N., a white male, age 52, with peptic ulcer; J. O., a white male, age 44 with inferior vena cava obstruction; A. P., a white female, age 79 with general arteriosclerosis and hypertensive vascular disease; and T. R., a white male, age 71 with general arteriosclerosis. The tenth case, B. S., a white male of 38 with syphilis and cerebral thrombosis, was under observation for 7 months.

The period of observation began in the late Fall of 1936 and extended to the Winter of 1937-1938. During this time the basic diet consisted of 350 grams of carbohydrate, 100 grams of protein and 115 grams of fat daily. The diabetics however were given 160 grams carbohydrate, 75 grams protein, and 90 grams of fat daily. The patients were weighed each week because of the possible bearing of the state of nutrition on the level of the serum lipids (8). It may be mentioned here that no significant fluctuation in weight took place in any case. Monthly blood counts were obtained and remained constant. The basal metabolic rate was determined each month and did not vary significantly except for an expected rise during thyroid administration. The serum

cholesterol of each patient was determined at weekly intervals by the method of Bloor, Pelkan, and Allen (2).

The plan of study for each case was similar. After a control period of about 2 months during which a base line for the serum cholesterol was established and observations were made on the variation of serum cholesterol from hour to hour the patient was given potassium iodide or thyroid for 4 to 6 weeks and the fat content of the diet was raised or lowered for a similar period. Following each of these experimental periods 4 or 5 weeks as a rule were allowed to pass to obtain further control observations. However, the high fat and low fat feeding periods were consecutive in order to heighten by continuity any effect secured. After the thyroid administration it was necessary to allow 4 to 6 weeks for the serum cholesterol to stabilize before beginning a control period, and the determinations made during these post thyroid weeks were discarded.

Figure 1 shows the serum cholesterol values for Patient T. R. during the entire period of study lasting for 62 weeks. It illustrates the temporal relationships in a typical case according to the plan just outlined.

Variation during 24 hour periods

It was stated above that, during the first control period when the fasting serum cholesterol was being determined for each patient at weekly intervals, opportunity was presented to perform repeatedly so-called 'cholesterol tolerance tests' upon the group of cases.

There are many conflicting statements in the literature as to the stability of the blood cholesterol values during a day with or without the ingestion of a high fat meal. The following reports should be regarded as typical but in no sense a complete survey of the subject.

Bruger and Somach (4) found an average standard deviation of ± 80 per cent in the whole blood cholesterol over a 24-hour period independent of the ingestion of food. McEachern and Gilmour (10) noted an individual variation of 25 to 84 mgm with an average of 40 mgm in 28 normal fasting humans over a

On the other hand, Boyd (1)

¹ Read by title before the American Society for Clinical Investigation May 2, 1938

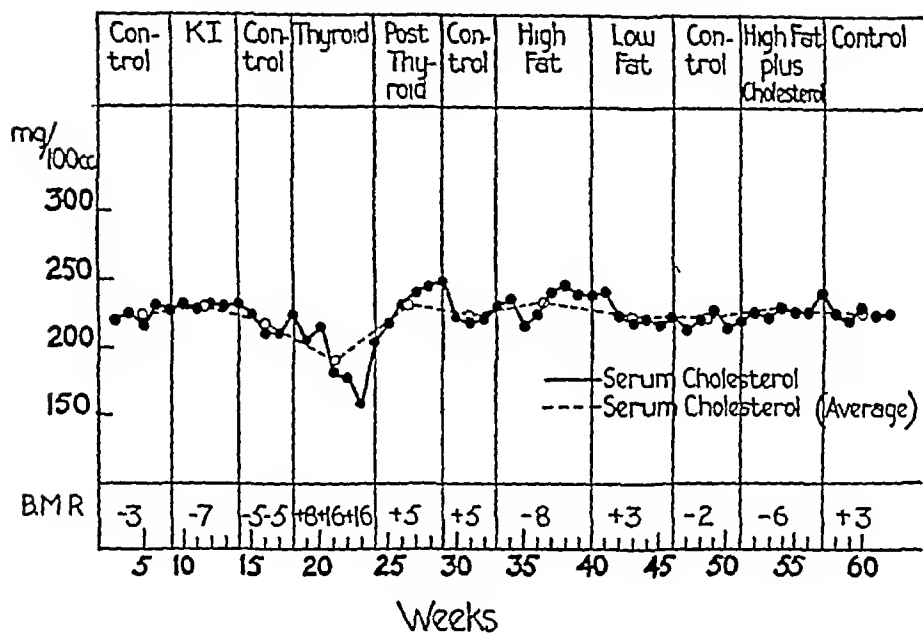


FIG 1 THE WEEKLY SERUM CHOLESTEROL LEVELS OF A SINGLE PATIENT, T R, DURING A PERIOD OF OBSERVATION OF 62 WEEKS

variations in an individual during a 24-hour period regardless of food, exercise, and so on

After a fat meal, Hiller, Linder, Lundsgaard, and Van Slyke (6) found no significant change in the plasma cholesterol of either normals or nephritics. Mjassnikow (11) detected no rise in blood cholesterol in normals or in patients with liver disease, but found an increase regularly in nephrotics. Blotner (3) reported that normal or malnourished individuals show no increase in plasma cholesterol after 500 cc of 20 per cent cream, but that a rise occurs in obese patients or those with diabetes insipidus.

In the present group of 10 cases, 44 tests were made as follows. A blood was taken for analysis at 8 a.m. with the patient in the fasting state. Breakfast consisted of fruit, 2 eggs, buttered toast, coffee, and 200 cc of milk to which 20 grams of cholesterol had been added. Dinner and supper were served at the usual hours. Additional bloods were taken at 10 a.m., noon, 4 p.m., and 8 a.m. of the following day.

The results of a single test on each of 5 patients are shown in Table I. They are typical of the whole group.

It is clearly shown that there was little or no change in the total serum cholesterol during the course of 24 hours. This tendency of the serum

TABLE I
Serum cholesterol during 24-hour periods

Patient	Total serum cholesterol (mgm per 100 cc)					Ester cholesterol (mgm per 100 cc)				
	8.00 a.m.	10.00 a.m.	12.00 M	4.00 p.m.	8.00 a.m.	8.00 a.m.	10.00 a.m.	12.00 M	4.00 p.m.	8.00 a.m.
T R	238	230	226	238	228	165	160	150	170	164
D C	241	236	233	242	241	180	152	166	165	174
H F	286	284	278	290	272	192	168	175	185	181
J O	255	240	256	245	250	165	179	160	188	186
F K	220	236	219	238	219	166	146	158	160	145

cholesterol to be maintained at a constant level during the day regardless of feeding was striking in each of the 10 patients studied. Such fluctuations as occurred were inconstant and apparently casual, resulting, perhaps, from errors in technique or from unknown causes. The possibility of their being the result of changes in hemoconcentration was investigated but no relationship could be demonstrated. The cholesterol ester varied more widely, but again according to no set pattern.

In order to broaden the scope of this phase of our observations, the same procedure was employed upon an additional group of 25 patients more acutely ill and representing a variety of disease entities. In these cases also it was ap-

parent that the serum cholesterol did not fluctuate significantly during the 24-hour period

Variation during a year

McClure and Huntsinger (9) analyzed 4 blood samples from each of 5 patients over a period of weeks or months and concluded that there was considerable variation in the cholesterol. This view was concurred in by Pucher and Sly (14) but was opposed by Robinson (15) as the result of a few observations on 2 cases. In a more extensive study, Schube (16) determined the whole blood cholesterol of 10 patients at weekly intervals for 16 weeks and found a maximum deviation of approximately 40 mgm from the mean for each case. Sperry (18) made 2 or more determinations of the serum cholesterol of 25 healthy adults at varying intervals, and found a maximum variation of 12.3 per cent from the average, while in 17 instances this variation was 6.2 per cent or less. From this he concluded, "In most, if not all, persons in health the amount of cholesterol in the serum appears to be maintained at a constitutional level which is characteristic for each individual and from which large deviations do not ordinarily occur."

In our series, the determination of the normal variation in the serum cholesterol was based on a study of 4 control periods. The first of these came at the beginning of the year's observation and lasted about 2 months. The second followed the period of potassium iodide administration and lasted 4 to 6 weeks. The third was of similar length and began 4 to 6 weeks after a period when thyroid was given. The fourth came at the end of the year following periods of high and low fat feeding and was 4 to 10 weeks long.

The level of the serum cholesterol for each individual tended to remain relatively constant during each of the 4 control periods, although these were separated by intervals of as much as 3 months. Based on the combined results for the 4 control periods, the first columns of Table II show the maximal range and average for each patient. When the single determination of the total serum cholesterol farthest from the average is considered, the variation ranges from 4 per cent (B S) to 15 per cent (H F) with less than 10 per cent in 6 cases.

TABLE II
Effect of KI and thyroid on serum cholesterol

Patient	Control periods				KI period				Thyroid period				Change in basal metabolic rate
	Total cholesterol		Factor	per cent	Total cholesterol		Factor	per cent	Total cholesterol		Factor	per cent	
	Range	Average			Range	Average			Range	Average			
	mgm. per 100 cc.	mgm. per 100 cc.		mgm. per 100 cc.	mgm. per 100 cc.		mgm. per 100 cc.	mgm. per 100 cc.		mgm. per 100 cc.	mgm. per 100 cc.		
D.G.	220-219	213	68	220-250	240	65	145-213	180	65	65	-3 to +14		
M.C.	338-401	368	71	212-378	303	62	210-400	323	64	64	-1 to +17		
M.C.	241-422	351	65	235-422	431	65	229-392	320	70	70	-7 to +17		
H.F.	237-308	280	86	270-280	280	80	204-250	236	78	78	+7 to +16		
F.K.	227-252	240	84	226-250	240	68	195-258	226	61	61	-7 to +18		
J.N.	277-341	308	70	302-321	320	60	200-321	254	68	68	0 to +14		
J.C.	244-290	265	70	235-250	266	68	184-227	206	62	62	-9 to +7		
A.P.	235-258	241	71	320-325	322	78	218-218	218	66	66	+8 to +28		
T.R.	210-232	222	64	228-322	271	87	158-216	190	66	66	-5 to +16		
B.S.	265-287	276	87	274-285	280	58	207-254	230	84	84	-10 to +20		

The levels of the cholesterol throughout the group average higher than has been usual in our experience. In 4 cases we should regard them as definitely above normal. The highest of these (M C) was a woman of 55 with well-controlled diabetes. M C, a male of 62 years, had hypertensive vascular disease, as did A P, a woman of 79. J N was a man of 52 with a healed peptic ulcer.

Effect of potassium iodide

Each patient was given 2 grams of potassium iodide daily for 4 to 6 weeks. This dose was well tolerated. There was no effect on the basal metabolic rate.

Table II shows the range and average of the total serum cholesterol and the average percentage of cholesterol ester. Similar figures for the control periods are given for comparison.

It is obvious that KI in this dosage for this length of time had no effect on the level of the total serum cholesterol or on the percentage of ester cholesterol in 9 of the 10 patients. In 1 case (M C), the female diabetic, the cholesterol values tended to be higher while KI was being given. The average for the KI period was 434 mgm compared with 384 mgm for the control periods. Whether or not the rise in serum cholesterol in this case is significant, we are unable to say.

Effect of thyroid

The administration of thyroid lasted 6 weeks in most cases. The dosage was determined by

the reaction of the patient and the rise in basal metabolic rate. The daily dose of thyroid varied from 30 to 240 mgm. The results are shown in Table II.

It will be noted that in every case the total serum cholesterol fell. This is seen not only in the averages for the period but more accurately by comparing the lower figure of the range with the range of values for the control periods. Without exception a new low value is established during thyroid administration. The percentage of cholesterol esters is apparently uninfluenced.

A fall in blood cholesterol has been noted previously with thyroid administration (5, 7, 13) although it has been emphasized that this fall does not occur in nephrosis (12, 17).

The basal metabolic rate was also increased in every case. This increase varied from 8 to 30 per cent with an average of 20 per cent.

When the thyroid administration was stopped, the serum cholesterol rose at varying rates toward control levels. Occasionally, it overshot previous values and then dropped back. During this stage of readjustment, which lasted 4 to 6 weeks, cholesterol determinations were discarded so far as the purposes of this paper were concerned.

Effect of high or low fat diets

Nine of the original 10 patients were available on whom to try the effect of diets high or low in fat. First they were given for 6 weeks a diet containing 300 grams of fat daily except in the case of the two diabetics whose basic diet of 160 C—75 P—90 F was changed to 100 C—75 P—200 F. The high fat intake was not pleasant, and it was with some difficulty that the patients were prevailed upon to carry through with it.

Without intermission the same patients were switched to a low fat diet containing less than 50 grams of fat daily. On this diet they remained for 6 weeks.

The results of the high fat and the low fat feeding on the serum cholesterol are shown in Table III.

Furthermore, at a later time, 3 of the patients again submitted themselves to the unpleasantness of the high fat diet, on this occasion supplemented daily by 10 grams of cholesterol in 200 cc. of

TABLE III
Effect of diet on serum cholesterol

Patient	Control periods			High fat diet			Low fat diet		
	Total cholesterol		Ester	Total cholesterol		Ester	Total cholesterol		Ester
	Range	Average		Range	Average		Range	Average	
	mgm. per 100 cc.	mgm. per 100 cc.	per cent	mgm. per 100 cc.	mgm. per 100 cc.	per cent	mgm. per 100 cc.	mgm. per 100 cc.	per cent
D.C.	220-240	233	68	209-260	238	69	225-260	239	70
M.C.	335-401	368	71	339-430	410	70	338-420	368	69
M.C.I.	344-432	384	65	339-410	388	74	365-420	391	76
H.F.	237-380	280	66	266-286	283	73	260-305	294	71
				294-300*	297	65			
F.K.	227-282	254	64	279-300	293	64	261-303	283	70
J.N.	277-341	308	70	312-351	332	69	299-354	328	70
J.O.	244-290	268	70	281-348	315	74	252-310	276	70
				280-294*	286	65			
A.P.	305-358	334	71	320-356	340	76	316-351	331	82
T.R.	210-232	222	64	215-246	234	72	216-241	223	70
				224-238*	228	53			

* High fat diet plus 10 grams of cholesterol daily

milk, for another 6-week period. The results of this are also incorporated in Table III where they may be distinguished by asterisks.

In 5 of the 9 patients there was no increase in the total serum cholesterol during the period of high fat feeding. In 4 cases a slight rise seemed to occur. The significance of this in 2 cases (F K and J N) was somewhat nullified by the failure of the cholesterol level to fall substantially on a restricted fat intake. In a third case (J O) the cholesterol did not show the same rise when the patient was again given a high fat diet with added cholesterol. Accordingly, it seems that too much emphasis should not be placed on these rises in serum cholesterol while on a high fat diet.

As for the addition of cholesterol to the high fat diet, it may be dismissed by saying that it was without effect when compared with the use of a high fat diet alone.

The total serum cholesterol values of patients on a low fat diet were in no wise different from those during the control periods.

With both diets, whether high or low in fat, the percentage of cholesterol ester rose slightly.

SUMMARY

1 The serum cholesterol of 9 patients was studied for 12 to 14 months, and in 1 case for 7 months.

2 The serum cholesterol of an individual tends to remain remarkably constant throughout the day,

regardless of the feeding of a breakfast rich in cholesterol. This same constancy for the individual is evident from week to week and month to month.

3 Potassium iodide given in doses of 2 grams per day for 4 to 6 weeks has no effect on the serum cholesterol level of the individual.

4 Thyroid administration produced a sharp drop in serum cholesterol in every case. This was accompanied by a rise in the basal metabolic rate.

5 A diet high in fat seemed to cause a slight rise of dubious significance in the serum cholesterol of 4 of 9 patients. This increase was not augmented by the addition of cholesterol to the diet in 3 cases.

6 A diet low in fat failed to influence the level of the serum cholesterol.

BIBLIOGRAPHY

- 1 Boyd, E. M., Diurnal variations in plasma lipoids. *J Biol Chem.*, 1935 110 61
- 2 Bloor, W. R., Pelkan K. F., and Allen D. M., Determination of fatty acids (and cholesterol) in small amounts of blood plasma. *J Biol Chem.* 1922, 52, 191
- 3 Bloetner H., Blood fat tolerance tests in malnutrition and obesity. *Arch Int. Med.*, 1935 55 121
- 4 Bruger M. and Somach, I., The diurnal variations of the cholesterol content of the blood. *J Biol Chem.*, 1932, 97 23
- 5 Duncan, A. G., The effect of thyroid administration on the blood cholesterol. *J Ment. Sc.* 1931 77, 332.
- 6 Hiller A., Linder G. C. Lundsgaard, C., and Van

- Slyke, D. D., Fat metabolism in nephritis. *J Exper Med.*, 1924 39 931
- 7 Lévy, M., and Lévy, M., Le traitement de l'hypercholestérolémie par la thyroxine. *Bull. Acad. de méd., Paris* 1931, 105, 666 (Chem. Abstr.)
- 8 Man, E. B., and Glidca, E. F., Serum lipoids in malnutrition. *J Clin. Invest.*, 1936, 15, 203
- 9 McClure, C. W., and Huntsinger M. E., Studies in fat metabolism. I. The influence on blood lipids of single foodstuffs. *J Biol. Chem.*, 1928 76, 1.
- 10 McEachern, J. M. and Gilmour, C. M., Studies in cholesterol metabolism I Physiological variations in blood cholesterol. *Canad. M. A. J.*, 1932, 26 30
- 11 Mjassnikow A. L., Über alimentäre Beeinflussung der Cholesterinämie beim Menschen. *Ztschr f klin. Med.*, 1926 103, 767
- 12 Page, I. H., and Farr L. E., The influence of high and low fat diets and thyroid substance on plasma lipids of nephrotic patients. *J Clin. Invest.*, 1936 15 181
- 13 Parhon, C. I., and Ornstein, I., Influence de la thyroxine sur la cholestérolémie et la lipémie. *Compt. rend. Soc. de biol.* 1931, 108, 303
- 14 Pucher G. W., and Sly G. E., Blood cholesterol. After fasting and after cholesterol ingestion. *Bull. Buffalo Gen. Hosp.*, 1929 7, 10
- 15 Robinson, R. H. O. B., Diagnostic value of the estimation of blood cholesterol in cholelithiasis. *Lancet*, 1929 2 540
- 16 Schube, P. G., Variations in the blood cholesterol of man over a time period. *J Lab. and Clin. Med.*, 1936, 22, 280
- 17 Schwarz, H., and Kohn J. L., Studies of nephritis in children. I Nephrosis. *Am. J. Dis. Child.*, 1922 24 125
- 18 Sperry, W. M., The concentration of total cholesterol in the blood serum. *J Biol. Chem.*, 1937, 117 391

PROTEINURIA FOLLOWING MOMENTARY VASCULAR CONSTRICTION

By LEON C. CHESLEY IRWIN MARKOWITZ, AND BENJAMIN B. WETCHLER
(From the Departments of Biochemistry and Urology, Margaret Hague Maternity Hospital,
Jersey City)

(Received for publication September 1 1938)

Vasoconstriction in the glomerular and pre-glomerular blood vessels of the kidney with a consequent anoxia in the capillaries has been hypothesized as a cause of many proteinurias, not only in non nephritic but even in acute nephritic conditions. The main evidence for this hypothesis is that many of the stimuli producing proteinuria are known also to interfere with the renal circulation, usually by causing vasoconstriction. However, because of the prolonged time element, and the administration of drugs, many of the earlier experiments have not been sufficiently swiftly executed to rule out possible causes other than vascular spasm *per se*. In some cases, vascular constriction, though probable, has not been shown to have occurred.

The literature is amply reviewed by Senator (1), Volhard (2), and Fishberg (3), and will not be detailed here. Some of the difficulties of interpretation will be merely indicated.

Johnson (4), Abesser (5), Christensen (6), and others found proteinuria in many of their subjects who had swum in cold water. Since cooling the skin causes renal vascular spasm, (Wertheim (7)), the proteinuria has been attributed to vasoconstriction. But Jehle (8) and Abesser (5) explain this proteinuria by the markedly lordotic posture assumed in swimming. Still another possibility is that the increased intra-abdominal pressure retards the venous return from the kidney, thereby causing proteinuria (3).

Schlombka (9) produced proteinuria in 9 of 37 subjects by immersing the lower parts of their legs in cold water for periods up to 25 minutes (average 16 minutes). He considered two possible causes, first, vascular spasm, and second, the local production in the chilled skin of some substance which affected glomerular permeability. He found that if the venous return from the legs was prevented, there was less proteinuria. Some subjects showed no effect from chilling alone, when the venous blood was occluded. This

might be considered as favoring the second explanation.

Starr (10) produced proteinuria by the administration of adrenalin and ephedrine. Unless there was a rise in blood pressure (vasoconstriction) no proteinuria resulted. Starr found proteinuria after CO₂ inhalation and hemorrhage, which also produce vasoconstriction. Cats excreted protein when excited. He believes that the vascular constriction causes an increase in the duration and extent of the normal intermittent interruptions in the glomerular circulation. Permeability of the glomerular membranes is so increased by these lengthened interruptions that when the blood flow is reestablished, protein escapes. Hermann (11) had long before shown that mechanical interruption of the renal blood flow results in proteinuria when the circulation is restored.

Orthostatic proteinuria seems to occur predominantly in individuals with labile vasomotor systems (3) and may depend upon the vascular instability (12).

In studying the vascular instability of patients with primary hypertension, Hines and Brown (13) have developed a "cold pressor test." This test consists in determining the subject's basal blood pressure after a period of rest in bed. One hand is then immersed in ice water for one minute. The blood pressure will usually rise abruptly and is nearly maximal within 30 seconds. The magnitude of the rise in pressure varies from patient to patient, and may be very marked in some individuals. Venous stasis does not prevent the effect which apparently is reflex. The abrupt increase in pressure with a rapid return to the basal level must, of course, depend upon transitory vascular constriction.

This response to the cold pressor test is given by many apparently normal individuals in whom no renal disease or hypertension can be found. The vascular spasm, in these subjects, occurs.

within 60 seconds and disappears within the next 60 seconds as shown by the excursion of the blood pressure. Here, then, is a means of producing a momentary vascular spasm. Will such a transient spasm be followed closely by proteinuria? If so, the experiments would be good evidence for the hypothesis that vascular spasm does result in protein leakage.

MATERIAL AND METHODS

In the course of a study of the cold pressor test in more than 500 pregnancies (14) it was found that in 12 per cent of all the women observed the blood pressure rose more than 25 mm Hg when the cold stimulus was given. Some of these "hyper-reactors" have been used in this investigation. The patients were in varying stages of pregnancy and puerperium, and a few were several months *postpartum* when the present experiments were made. A control series of patients giving blood pressure rises of less than 20 mm Hg was utilized. In both groups, patients were selected both with and without pre-existing proteinuria, with and without toxemia of pregnancy, and with and without demonstrable renal disease.

During the morning of the test, the patients were allowed to have breakfast, and were encouraged to take copious amounts of fluids. Ureteral catheterization was then done by one of us. Indigo carmine was injected intravenously, and the efficiency of the kidneys in excreting it was noted. The procedure was explained to all of our patients in order to minimize nervous tension. No sedative drugs were used.

On returning to bed, the patients were left for varying periods until the blood pressure had become stabilized at an apparently basal level. Two urine specimens were then obtained from each ureteral catheter, usually over 4-minute periods, these served as the controls. The hand was then immersed up to the wrist in ice water for one minute, while the blood pressure was taken at 30 and 60 seconds, and every minute thereafter. Successive urine collections were then made, in graduated centrifuge tubes, at 4-minute intervals. In many cases, the venous return from the chilled hand was prevented by a tourniquet.

Grossly bloody urine was discarded. The urine for analysis was centrifuged, first at 1200, then at 2500 r.p.m. for about 30 minutes. Five-tenths to 2 ml. of the supernatant urine was then pipetted into centrifuge tubes, diluted to about 10 ml with distilled water, and the protein precipitated by the addition of 2 ml. each of 10 per cent sodium tungstate and $\frac{2}{3}$ N sulfuric acid (15). After standing overnight, the tubes were centrifuged and the supernatant fluid drained off. The protein was redissolved in 10 per cent sodium tungstate, and the precipitation repeated. The following day, the protein was dissolved in dilute sodium hydroxide and transferred quantitatively to Folin nonprotein nitrogen tubes. Digestion was accomplished by the Wong persulphate method (15), and the digest nesslerized directly and read

colorimetrically against standards containing from 0.05 to 0.25 mgm of nitrogen.

In some cases, an approximate measurement of the glomerular filtration was attempted by determining the plasma clearance of endogenous creatinine (16). The creatinine of both plasma and urine was determined by the methods of Folin (17).

RESULTS

The subjects used in this study have been divided into two groups, those who gave no response in proteinuria and those who did. The findings for the first group are abstracted in Table I. These 24 patients, with 2 exceptions,

TABLE I

*Proteinuria in control series of patients who showed either no proteinuria or no increase in proteinuria after the cold pressor test**

Patient number	Diagnosis	Blood pressure rise mm Hg	Protein concentration †			Minute excretion of protein †		
			Control	Maximal	Period	Control	Maximal	Period
1-5 R & L	Normal	—4/—4	0.0	0.0		0.000	0.0	
6-9 R & L	Toxemia	16/16						
10 R	Normal	12/20	0.0	0.0		0.000	0.0	
11 R	Toxemia	10/12	57.3	75.2	3	0.420	0.510	3
12 R	Pyelitis	14/20	52.0	56.0	4	0.210	0.200	4
13 R	Normal	12/14	35.0	30.8	2	0.099	0.900	1
14 L	Nephritis	6/15	491.0	750.0	4	1.470	1.500	4
15 R	Toxemia	10/10	290.0	316.0	2	0.945	0.942	3
15 L	Toxemia	10/10	626.0	752.0	3	1.410	1.500	3
16 R	Hyper-tension	4/0	0.0	0.0		0.000	0.000	
16 L	tension	4/0	25.0	24.0	4	0.204	0.214	3
17 R	Toxemia	10/8	169.0	303.0	3	0.796	0.606	3
18 R	Toxemia	20/16	106.0	92.0	1	0.286	0.276	1
18 L	Toxemia	20/16	129.0	115.0	2	0.348	0.354	4
19 R	Toxemia	16/16	53.0	32.2	4	0.480	0.350	4
19 L	Toxemia	16/16	73.4	28.1	4	0.350	0.310	4
20 L	Nephritis	10/13	114.0	250.0	2	0.510	0.380	1
21 R	Normal	18/10	31.9	58.6	4	0.266	0.281	4
22 R	Toxemia	10/20	256.0	268.0	4	0.724	0.642	4
22 L	Toxemia	10/20	271.0	268.0	1	0.804	0.890	1
23 R	Hyper-tension	28/42	32.0	49.0	4	0.288	0.262	3
23 L	tension	28/42	38.4	38.0	3	0.442	0.430	2

* Only two of these gave rises of more than 16/16 mm in blood pressure.

† "Control" is average of two urines taken before cold stimulus. "Period" refers to the 4-minute interval in which observation is recorded. "R" stands for the right kidney and "L" for the left. "Maximal" is the highest proteinuria observed after the administration of the cold stimulus.

had but small increases in blood pressure following the cold pressor test, none showed any increase in protein excretion. In some cases the protein concentration in the urine increased, but this was counterbalanced by decreased urine volume. The changes in minute volume output of urine seem to be of no significance. In some cases marked increases in volume occurred, in

some marked decreases, while in many the variations were only slight. The subjects in this group were all women ranging from the third month of pregnancy to the third month after delivery. Some were normal pregnancies, some were toxemic. One patient had pyelitis and three were thought to have permanent hypertension. One third of the group had no proteinuria at all prior to the test, while the other two-thirds had some degree of proteinuria ranging from faint traces to 5 grams per liter.

The patients of this first group may be taken as controls, they gave only slight vascular constriction, as shown by the blood pressure rises which average 9/10 mm Hg systolic/diastolic, and following the stimulus there was no increased proteinuria.

In Table II are shown the significant results obtained in 23 equally diverse subjects whose blood pressure rises were in excess of 16/16

TABLE II

Proteinuria in series of patients who gave more marked responses to the cold pressor test. All other conditions as in control series. Column heads and abbreviations as in Table I.

Patient number	Diagnosis	Blood pressure rise	Protein concentration			Minute excretion of protein		
			Control	Maximal	Period	Control	Maximal	Period
		mm. Hg		mgm. per cent		mgm.	mgm.	
25 R.	Pyelitis	16/20	28.0	117.0	4	0.219	0.745	4
26 R.	Normal	40/46	30.0	102.0	3	0.268	0.902	3
27 R.	Normal	30/20	29.0	82.0	3			
28 L.	Normal	28/26	45.0	150.0	4	0.724	3.220	4
29 R.	Normal	38/40	268.0	500.0	4			
29 L.	Normal	38/40	122.0	376.0	2			
30 R.	Pyelitis	22/24	42.0	134.0	2			
31 L.	Normal	26/30	80.0	100.0	2	0.800	0.780	2
32 R.	Toxemia	16/24	00.0	36.0	2	0.000	0.279	2
32 L.	Toxemia	16/24	00.0	00.0	2	0.000	0.000	2
33 R.	Normal	52/7	16.8	88.2	2	0.174	0.590	2
33 L.	Normal	52/7	16.8	23.4	2	0.207	0.330	2
34 R.	Nephritis	28/24	67.2	114.0	2	0.154	0.863	2
34 L.	Nephritis	28/24	234.0	230.0	4	1.100	0.815	2
35 L.	Toxemia	40/40	13.2	41.5	4	0.103	0.454	3
36 R.	Normal	26/24	16.2	76.2	3	0.143	0.288	2
36 L.	Normal	26/24	25.4	73.0	4	0.510	0.342	2
37 R.	Nephritis	24/30	274.0	576.0	3	0.438	0.892	2
37 L.	Nephritis	24/30	363.0	816.0	4	1.268	1.700	3
38 R.	Normal	30/40	287.0	628.0	4	0.660	1.450	2
39 R.	Normal	72/75	44.0	55.0	3	0.000	0.164	3
39 L.	Normal	72/75	80.0	121.0	3	0.128	0.340	3
40 R.	Normal	20/34	20.9	24.0	4	0.233	0.340	4
40 L.	Normal	20/34	06.0	59.0	4	0.000	0.442	4
41 L.	Toxemia	22/15	149.0	185.0	3	0.344	0.684	4
41 L.	Toxemia	22/15	198.5	269.0	3	2.210	2.580	2
42 L.	Toxemia	18/16	588.0	957.0	4	0.707	3.080	2
43 R.	Normal	24/20	85.6	136.4	4	0.269	0.541	2
43 L.	Normal	24/20	168.0	384.0	3	0.274	0.488	3
44 R.	Normal	43/45	68.8	108.0	2	0.868	1.342	2
44 L.	Normal	43/45	35.9	50.2	3	0.193	0.262	2
45 R.	Normal	28/22	73.0	72.5	2	0.990	0.980	1
46 L.	Normal	22/20	33.2	56.0	2	1.077	1.410	3
47 R.	Toxemia	34/30	59.5	94.5	4	0.164	0.449	2
Mean		29/30	112.5	180.0	4	0.517	0.853	2

All of these patients showed an increase in proteinuria subsequent to the application of the cold stimulus. (Two patients giving such blood pressure rises did not excrete increased amounts of protein; they are included in Table I.) In 3 cases there was no preexisting proteinuria but following the blood pressure rise, proteinuria appeared. In the other 20 cases, the original protein excretion was increased. Five subjects developed proteinuria from one kidney but not from the other. In these 23 patients 36 kidney urine series were analyzed. All but 5 series showed increased concentration of protein. Six failed to show an increased excretion (volume \times concentration). Of these 6 series, in 5 cases one kidney gave an increased proteinuria while the other did not. In the sixth case the urine from only one kidney was analyzed.

The average responses in the minute urine volume output, urine protein concentration, and minute excretion of protein are shown in Figure 1.

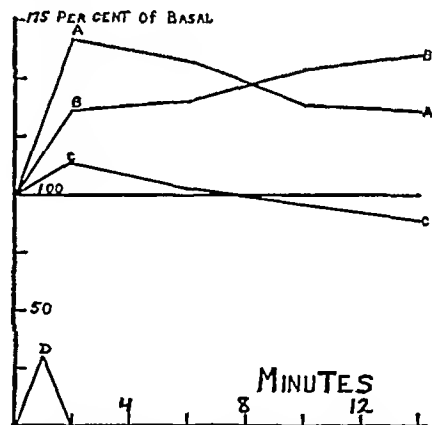


FIG. 1 THE CHANGES IN URINE VOLUME OUTPUT, URINE PROTEIN CONCENTRATION, AND MINUTE OUTPUT OF PROTEIN FOLLOWING VASCULAR CONSTRICTION.

A = Minute excretion of protein
B = Concentration of urine protein
C = Urine volume output

All are plotted as percentages of the basal (control) levels, which are taken as 100 per cent.

D = Average change in t
Absolute values are given

It is barely possible that this production of proteinuria is more apparent than real. For instance, it might be argued that not all of the urine drains through the ureteral catheters in the control periods, some escaping down the ureters. This would give false low basal proteinurias. Following the cold stimulus, the ureters might contract with a consequent diversion of more urine to the catheters, thereby apparently increasing the protein excretion. This cannot be the case in the three instances where proteinuria appeared for the first time after the cold stimulus was applied (32R, 38L, and 40L, Table II).

To control the possibility that incomplete collections of urine were vitating the results, a series of experiments was done in which the proteinuria was calculated on the basis of the minute excretion of creatinine. If the urine collections were complete, this relates the protein excretion to the glomerular filtration (16). If the urine collections were not quantitative, the error of collection is neutralized by comparing the proteinuria with the creatinine excretion, since the ratio gives the relative amounts of protein and creatinine filtered. Table III shows the results of the experiments in which simultaneous measurements of protein and creatinine excretion were made. This ratio of protein excretion to creatinine excretion is independent of errors in urine collection and establishes the validity of the conclusion that vascular constriction is followed by increased protein leakage. In 8 of 12 cases,

the ratios denoting the relative filtration of protein are markedly increased, while in 2 others they are slightly increased. The averages are represented graphically in Figure 2.

Assuming accurate collection of urine, the fluid volume filtered seems to diminish following the vascular constriction produced by the cold stim-

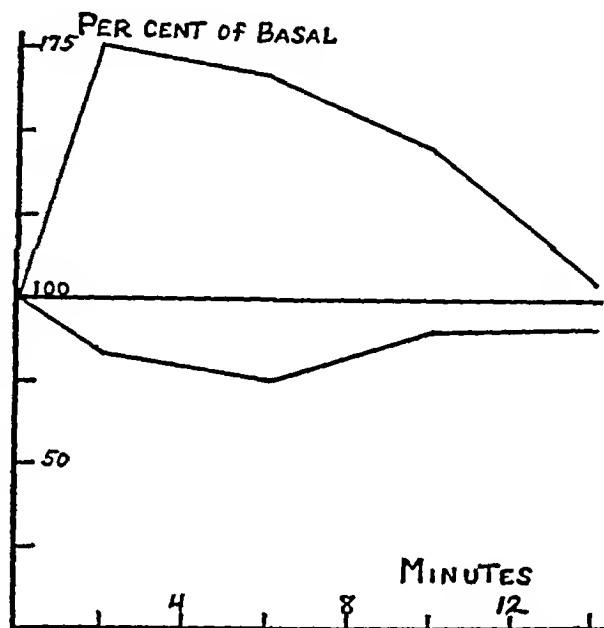


FIG 2 THE CHANGES IN GLOMERULAR FILTRATION (LOWER CURVE) AND IN THE PROTEIN EXCRETED PER UNIT OF GLOMERULAR FILTRATION (UPPER CURVE)

Both curves plotted as percentages of basal (control) levels which are taken as 100 per cent. Absolute values are given in Table III.

TABLE III

The increased glomerular filtration of urine protein in subjects giving appreciable blood pressure rises after cold stimulus

Patient number	Minute excretion of protein					Minute excretion of creatinine					Protein/creatinine				
	Control	1	2	3	4	Control	1	2	3	4	Control	1	2	3	4
	mgm	mgm	mgm	mgm	mgm	mgm	mgm	mgm	mgm	mgm					
23 L	0.442	0.253	0.357	0.430	0.380	0.311	0.266	0.292	0.402	0.405	1.42	0.96	1.22	1.07	0.94
39 R	0.196	0.470	0.220	0.264	0.402	0.310	0.672	0.350	0.310	0.942	0.63	0.70	0.63	0.82	0.42
40 R	0.235	0.312	0.270	0.308	0.340	0.405	0.350	0.335	0.350	0.340	0.58	0.89	0.81	0.88	1.00
40 L	0.000	0.000	0.245	0.405	0.442			0.275	0.233	0.162	0.00	0.00	0.89	1.74	2.72
41 R	0.344	0.436	0.626	0.659	0.684	0.235	0.230	0.207	0.211	0.246	1.46	1.90	3.03	3.12	2.78
43 R	0.369	0.251	0.341	0.300	0.432	0.150	0.091	0.130	0.116	0.145	2.46	2.75	2.62	2.59	2.97
43 L	0.274	0.395	0.252	0.488	0.319	0.085	0.072	0.043	0.058	0.048	3.22	5.52	5.84	8.44	6.59
44 R	0.878	1.340	0.920	0.974	0.765	0.379	0.362	0.213	0.355	0.298	2.32	3.71	4.32	2.74	2.57
44 L	0.139	0.242	0.169	0.094	0.167	0.104	0.183	0.167	0.072	0.154	1.34	1.32	1.01	1.31	1.09
45 R	0.990	0.980	0.594			0.324	0.226	0.204			3.06	4.33	2.91		
45 L	1.678	5.560	4.310	2.760	2.150	0.422	0.373	0.386	0.443	0.468	4.00	14.90	11.17	6.24	4.58
46 L	1.077	1.680	1.060	1.410	1.036	0.414	0.375	0.297	0.354	0.304	2.61	4.48	3.57	3.99	3.41
Average	0.552	0.993	0.780	0.736	0.647	0.285	0.291	0.242	0.264	0.319	1.935	3.410	3.222	2.790	2.030

vascular spasm Brodie (18), in computing the force necessary to propel the urine down the renal tubules, has presented the following measurements of the various parts of the dog's nephron. Each dimension cited is the grand average of many measurements made in several dogs. An approximation to the volume capacity of a nephron may be obtained by calculation from the formula for a cylinder, and thus the "dead space" may be roughly estimated (Table V)

TABLE V

Dimensions of the dog's nephron, as calculated from Brodie's (18) data

Portion	Length	Diameter	Capacity
	mm	micra	c mm
Proximal convoluted tubule	11.0	12.0	0.001244
Descending loop of Henle	8.5	10.0	0.000668
Ascending loop of Henle	8.5	9.5	0.000541
Distal convoluted tubule	2.0	18.0	0.000509
Collecting tubules	22.0	12.0	0.000237
Total capacity of nephron and collecting tubule			0.003199

This volume capacity of the nephron which we have calculated is widely variable, depending upon the degree of diuresis as Brodie showed, and probably also upon the intrarenal venous blood pressure. Furthermore, errors in the measurement of diameters are squared in the calculation of volume. Brodie's measurements were made in histological sections, and probably do not represent accurately the living condition.

According to Smith's (19) recent review of literature on the kidney, there are about 1,000,000 nephrons in each human kidney. This gives a value of 3.2 ml as an approximation to the fluid volume capacity of the renal tubules, or "dead space." On the usual assumption that about 60 per cent of the nephrons are active at any given time, one would expect that from the moment that protein appears in the glomerular filtrate until it reaches the pelvis of the kidney, 1.92 ml of protein-free urine must be passed. To this volume we must add 0.35 ml, the average capacity of a number 5 catheter, giving a volume of 2.27 ml. There is still an undetermined fluid volume between the area cribrosa and the opening of the catheter in the pelvis. Roughly, we might estimate the total dead space, from the glomerulus to the tube in which the urine was collected, as about 3.0 ml.

Crude as this calculation may be, there is another consideration which supports the result obtained. Smith, Goldring, and Chasis (20) have noted the time after intravenous injection of phenol red at which the dye appears in the bladder, with different urine flows. When the urine volume output is about one ml per minute, the dye appears in 200 seconds. Allowing 26 seconds for the circulation time, this gives 2.9 ml as the "dead space" for the human kidney.

These volumes are in excellent agreement with the observed urine volumes in the three cases (32R, 38L, and 40L) where there was no basal proteinuria, and protein did later appear. Measuring from the end of the cold stimulus (at which time the vascular spasm begins to relax) until the beginning of the collection period in which protein appeared in the urine, we find that an average of 2.7 ml of protein-free urine had been collected. The individual volumes were 2.3, 2.6, and 3.1 ml. This would indicate that the protein must escape from the capillaries at a time very close to the restoration of the blood flow through the glomerulus, which occurs with the release of the vasospasm.

It is interesting to calculate how much protein leaks through the capillaries, and in what concentration, following the momentary vascular spasm. We shall take two extreme cases from Table II, 32R and 37L. The glomerular filtration for the one kidney will be assumed to be somewhere between 50 and 60 ml per minute. In 32R, with a urine volume of 0.775 ml per minute, the protein concentration in the only specimen to show protein was 36 mgm per cent. The total amount of protein excreted here, following the vascular spasm, was 1.116 mgm. The urine was concentrated from 65 to 78 times, say 72. Therefore the concentration of protein in the glomerular filtrate was $36/72 = 0.50$ mgm per 100 ml. In 37L the basal proteinuria was high—362 mgm per cent, and rose to 816 mgm per cent after the cold stimulus, an increase of 454 mgm per cent. The urine volumes were 0.35 and 0.25 ml per minute, giving concentration ratios of about 150 and 210. Therefore, the concentrations of protein in the glomerular filtrate, before and after the cold stimulus, would be 2.42 and 3.88 mgm per cent, an increased

protein leakage into the glomerular filtrate of about 1.46 mgm per 100 ml

From the calculation, it is seen that the effect of the 'momentary spasm upon the capillary permeability is not really very marked. It appears to be greater because of the fact that the fluid filtered by the capillaries is concentrated many times over by the kidney tubules. Apparently the factor which causes the proteinuria is a sudden intensification of the vascular constriction. Severely hypertensive patients may have protein-free urines and in several of our cases moderate hypertension without proteinuria were observed. When, however, the blood pressure was abruptly driven up from whatever basal level, proteinuria appeared. This was observed by us in a man who has had a hypertension of several years' duration. In his case, voided urines were used, and the vascular constriction was produced by having him walk about after a half hour's rest. The basal blood pressure was 150/110 after walking about it was 208/138. Meanwhile the urine, which was protein free in the rest period, suddenly showed protein (4 mgm per cent), and the volume output fell by about 50 per cent. The proteinuria which appeared was slight, possibly because of the renal vascular sclerosis. Such sclerosis is probable in this case since the urinary specific gravity is fixed at 1.010. Conceivably, in hypertension the capillaries are either not markedly anoxic or have become adjusted as judged by their permeability to the existing degree of anoxia. When further constriction is superimposed with reduction in blood flow and oxygen supply, the permeability becomes impaired and protein leakage ensues.

One possible criticism of the results may be mentioned here. Ureteral catheterization, in some subjects, causes a transitory proteinuria (3). Since such catheterizations were part of the routine procedure, it should be emphasized that the patients were returned to bed after catheterization, and left there for at least 30 minutes before beginning the test. Furthermore, two control urine specimens were obtained from each ureteral catheter before the cold stimulus was given.

SUMMARY AND CONCLUSIONS

Forty-seven subjects were used in a study of the relation of proteinuria to vasospasm. The

subjects were women ranging from the third month of pregnancy to the ninth month after delivery. Some had normal pregnancies, some toxic. Some had a preexisting proteinuria, others had not, some had demonstrable renal disease, while the majority had not.

Urine was collected at short intervals from ureteral catheters, during which time vasospasm was produced by immersing one hand in ice water for one minute. As a result of this procedure, the vascular constriction appears and disappears within 120 seconds.

Twenty four patients showed no increase in urinary protein excretion following the cold stimulus. With two exceptions, none of these had rises in blood pressure of more than 16/16 mm. Hg, that is, vasospasm was slight. These patients are regarded as the control series.

Twenty three patients responded with an increased excretion of urinary protein immediately following the cold stimulus. All of these subjects showed blood pressure rises of more than 16/16 mm. Hg, that is, vasospasm was definitely produced.

The increased proteinuria of the second series actually occurs, as shown by the protein excretion/creatinine excretion ratio. This ratio indicates that after vascular constriction more protein escapes per unit of glomerular filtration. Incomplete collections of urine volume would not vitiate the conclusion drawn from this ratio.

The right and left kidney usually reacted similarly. In a few cases one kidney showed increased proteinuria while the other did not.

Changes in pulse pressure apparently do not influence the appearance of proteinuria induced by the cold stimulus.

It is concluded from the promptness of reaction, independence of venous return from the chilled hand, and from calculations of the renal tubular volume capacity, that the proteinuria begins with the release of the vascular spasm.

We are indebted to Drs. S. A. Cosgrove, J. F. Norton and E. G. Waters for their permission to use patients from their services. Dr. Cosgrove also read and criticized the typescript. Some of the ureteral catheterizations were done by Dr. Dana Cox and Dr. E. W. Cartwright. Most of the patients giving marked blood pressure responses to the cold pressor test were selected by Mrs. E. R. Chesley from her large clinic series.

BIBLIOGRAPHY

- 1 Senator, H, Die Albuminurie in physiologischer und klinischer Beziehung und ihre Behandlung August Hirschwald, Berlin, 1890, 2d ed
- 2 Volhard, F, Nieren und Ableitende Harnwege. VI Die Albuminurie. Handb. d. inn. Med., Julius Springer, Berlin, 1931, 6, part I, 812
- 3 Fishberg, A. M., Hypertension and Nephritis, Lea and Febiger, Philadelphia, 1934, 3d ed
- 4 Johnson, G, Latent albuminuria its etiology and pathology Brit. M. J., 1879, 2, 928
- 5 Abesser, Sportärztliche Beobachtungen bei der Schutzpolizei München. med. Wchnschr., 1926, 73, 1697
- 6 Christensen, H. B., Untersuchungen des Urinsedimentes von Sportsleuten und Nephritikern Deutsches Arch. f. klin. Path., 1909, 98, 379
- 7 Wertheim, De l'influence de la Refrigeration de la Peau sur la Circulation du Rein. Arch. d. Path., 1894, 308 (Cited by Schlombka (9))
- 8 Jehle, L., Die Albuminurie. Klinische und experimentelle Beiträge zur Frage der orthostatisch-lordotischen und der nephritischen Albuminurie. Julius Springer, Berlin, 1914
- Schlombka, G., Untersuchungen über den Einfluss äusserer Abkühlungen auf die Nierentätigkeit. Ztschr. f. d. ges. exper. Med., 1928, 61, 405
- Starr, I., The production of albuminuria by renal vasoconstriction in animals and in man J. Exper. Med., 1926, 43, 31
- Hermann, M., Sitzungsber. k. akad. Wissensch., Math.-Naturwissensch. cl., Wien, 1862, 45, part 2, 317 (Cited by Starr (10))
- 12 Erlanger, J., and Hooker, D. R., An experimental study of blood pressure and of pulse pressure in man, including a consideration (c) of the relation between blood pressure and pulse pressure and the output of albumin in a case of orthostatic albuminuria. Johns Hopkins Hosp. Rep., 1904, 12, 346
- 13 Hines, E. A., and Brown, G. E., A standard test for measuring the variability of blood pressure its significance as an index of the prehypertensive state. Ann. Int. Med., 1933, 7, 209
- 14 Chesley, L. C., and Chesley, E. R., The cold pressor test as a means of predicting toxemia of pregnancy (In preparation)
- 15 Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry Vol. II Methods Williams and Wilkins Co., Baltimore, 1932, pp. 528 and 682
- 16 Miller, B. F., and Winkler, A. W., The renal excretion of endogenous creatinine in man Comparison with exogenous creatinine and inulin. J. Clin. Invest., 1938, 17, 31
- 17 Hawk, P. B., and Bergeim, O., Practical Physiological Chemistry Blakiston's Sons, Philadelphia, 1931, 10th ed., pp. 421 and 835
- 18 Brodie, T. G., A new conception of the glomerular function Proc. Roy. Soc. London, s. B, 1913, 87, 571
- 19 Smith, H. W., The Physiology of the Kidney Oxford University Press, New York, 1937, pp. 67 and 262
- 20 Smith, H. W., Goldring, W., and Chasis, H., The measurement of the tubular excretory mass, effective blood flow and filtration rate in the normal human kidney J. Clin. Invest., 1938, 17, 263

CLINICAL STUDIES OF THE BLOOD VOLUME. V HYPERTHYROIDISM AND MYXEDEMA

By JOHN G GIBSON, 2d AND ALFRED W HARRIS

(From the Department of Medicine Harvard Medical School and the Medical Clinic of the Peter Bent Brigham Hospital Boston)

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In a study of the blood volume in normal human beings (1) a relationship between the total circulating blood volume and the basal metabolic rate was observed. In a group of 99 individuals the distribution of cases above and below the average total blood volume was similar to the distribution of basal metabolic rates above and below average normal values, and the decline in total blood volume with advancing age was parallel to the decline in basal metabolic rate. An increase in total blood volume, the degree of which was related to the severity of the condition, was found in patients with congestive heart failure (2) in which it may be assumed that the oxygen carrying mechanism was inefficient in meeting the tissue oxygen requirement. This observation suggested that the level of oxygen consumption might exert a considerable effect on the circulating blood volume, and prompted us to undertake a study of the blood volume in hyperthyroidism and myxedema in which the metabolism is severely disturbed.

Previous studies have indicated that hyperthyroidism is characterized by an abnormally high (3, 4, 5) and myxedema by an abnormally low circulating blood volume (6, 7, 8). Chang (4), using the CO method, found no direct relationship between the degree of increase in basal metabolic rate and increase in blood volume but observed decreases in blood volume with clinical recovery in all his cases. Goldbloom and Libin (5), using a dye method, found the increases above their normal values in hyperthyroidism great enough to lead them to believe that determination of blood volume was of value in the differential diagnosis of hyperthyroidism. Thompson (6) found the plasma volume about 30 per cent below normal in untreated cases of myxedema. On treatment with the return of the basal metabolic rate to normal, the plasma volume rose to within normal limits.

MATERIAL AND METHODS

Twenty females and 5 males with clinically proven hyperthyroidism were studied. The basal metabolic rate was more than 15 per cent above normal in all cases. In 5 males and 10 females the changes in plasma and total blood volume were followed during the course of therapy and determinations were made in all of these cases at intervals varying from 5 to 22 days after subtotal thyroidectomy was performed. In 2 males and 4 females blood volume was determined pre-operatively after the administration of Lugol's solution.

Single blood volume determinations were made in 5 females and 2 males with clinically proven myxedema. The basal metabolic rate was more than 15 per cent below normal in all cases. One man with pernicious anemia developed myxedema during a relapse, and another man developed myxedema some time after subtotal thyroidectomy.

Basal metabolic rates were determined by the standard technique of Benedict and Roth (9), plasma and total blood volume were determined by the direct method of Gibson and Evans (10), venous pressure was determined by the direct method of Evans (11) and circulation time by the decholin method of Winternitz, Deutsch, and Brull (12).

RESULTS

Surface area rather than height was taken as the basis for prediction of normal plasma and total blood volume inasmuch as the basal metabolic rates were based upon surface area. In almost every case the clinical history revealed the loss of some weight prior to admission to the hospital, and it is possible that the predicted volumes based upon surface area may represent values that were too high in some instances. The actual total blood volumes determined in these cases may well represent, therefore, greater percentage deviations from the normal volumes than indicated.

The course of the basal metabolism, plasma and total blood volume, hematocrit, venous pressure and circulation time in 25 cases of hyperthyroidism and in 7 cases of myxedema is shown in Table I.

TABLE I

Absolute plasma, cell, and total blood volume, normal total blood volume predicted from surface area, venous pressure, and circulation time in 25 cases of hyperthyroidism and 7 cases of myxedema

Case number	Date	Sex	Age	Height	Weight	Surface area	Predicted total blood volume	Venous pressure	Circulation time	Basal metabolic rate	Blood volume			Hematocrit	Percentage deviation from predicted total blood volume		Remarks
											Plasma	Cell	Total blood		+	-	
			years	cm.	kgr.	square meters	cc.	mm. H ₂ O	seconds	per cent	cc.	cc.	cc.	per cent of cells			
25 PATIENTS WITH HYPERTHYROIDISM																	
9	December 10 1934	F	40	160.0	55.0	1.55	4025	90	8	+27	2350	1970	4350	45.3	8.1		
12	December 13 1934	F	23	163.6	66.0	1.71	4175	80	11	+55	3105	1665	4770	38.1	14.3		
18	April 8, 1935	F	42	156.8	51.7	1.49	3850	80	10	+51	2350	1650	4000	41.6	3.9		
23A	May 4, 1935	F	41	152.5	66.0	1.50	3900	75	10	+61	2335	1575	3910	40.7	0.3		
23B	May 24 1935			152.5	66.4	1.515	3925	90	10	+3	2270	1200	3470	36.1		11.6	8 days postoperatively
25A	May 17, 1935	F	34	167.5	54.5	1.60	4100	70	9	+47	2740	1610	4350	37.4	6.1		
25B	June 15 1935			167.5	52.4	1.58	4075	65	10	+6	2650	1345	4025	34.0		1.2	10 days postoperatively
31A	May 22, 1935	M	22	164.0	43.2	1.40	3450	55	11	+70	2180	1900	4380	42.4		27.0	
31B	June 12, 1935			164.0	44.6	1.44	3725	105	11	+7	2250	1590	3840	41.8	3.1		8 days postoperatively
60A	July 8, 1935	F	38	163.8	70.0	1.75	4175	60	10	+46	2665	1765	4430	40.5	6.1		
60B	July 25, 1935			163.8	64.6	1.69	4175	55	11	-16	2240	1540	3780	40.8		8.8	9 days postoperatively
40	June 17, 1935	F	28	158.8	48.4	1.47	3800	100	8	+66	1900	1760	3660	38.2		3.7	
64A	July 13, 1935	F	48	157.4	52.3	1.50	3900	65	12	+59	2170	2160	4630	37.5	18.7		
64A	September 20 1935	F	42	156.4	62.4	1.61	4100	60	12	+35	3090	2000	5090	39.3	24.1		
64B	September 30 1935			156.4	60.0	1.59	4075	65	12	+31(1)	2535	1675	4210	39.9	3.3		Iodine administration
64B	October 1 1935	F	28	171.5	50.6	1.58	4075	65	11	+30	2270	1600	3870	41.5		5.0	
64B	October 15, 1935			171.5	50.0	1.57	4050	65	12	+11	1990	1330	3320	41.2		17.9	7 days postoperatively
64B	May 12 1936	F	37	162.8	52.0	1.53	3950	30	9	+20	2265	1365	3630	39.8	8.1		
33A	September 11 1936	M	36	174.5	65.3	1.78	5250	75	14	+24	2600	2220	4820	46.0		8.2	
33B	September 23 1936			174.5	65.5	1.79	5275	45	14	+3	2980	2380	5360	44.4		1.6	Iodine administration
33C	October 16, 1936			174.5	66.8	1.79	5300	20	18	+3	2900	2225	5125	43.4		3.3	22 days postoperatively
232A	September 11, 1936	F	28	152.5	47.6	1.41	3525	90	12	+38	2075	1435	3510	40.7		9.4	
232B	September 23, 1936			152.5	55.8	1.51	3925	80	12	+14	2150	1320	3470	37.9		9.2	Iodine administration
232C	October 16, 1936			152.5	54.1	1.49	3575	100	17	-19	2160	1135	3315	34.3		11.5	21 days postoperatively
241A	October 22, 1936	M	39	176.5	63.7	1.78	5250	40	16	+17	2955	2495	5450	45.8		3.8	
241B	November 2, 1936			176.5	60.3	1.61	5350	40	22	-4	2530	2000	4530	44.3		15.3	Iodine administration
335A	March 1 1938	F	44	172.7	76.4	1.89	4175	75	19	+23	2600	2160	4760	45.5		14.0	
340	March 21 1938	F	63	166.3	74.4	1.81	4175		18	+46	2900	2040	4940	41.1		18.3	
344	March 30 1938	F	39	171.5	85.9	1.97	4175		14	+30	2810	2060	4870	42.3		16.6	
345A	April 1, 1938	F	39	162.5	60.6	1.63	4125		10	+48	2350	1650	4000	41.2		3.0	
345B	April 20 1938			162.5	59.3	1.62	4100		8(7)	+28	2000	1530	3530	43.4		13.0	Iodine administration
346A	April 12, 1938	F	52	158.8	57.0	1.57	4025		13	+47	2160	1580	3760	41.9		6.5	
346B	April 29 1938			158.8	55.4	1.55	4000		13	+1	2220	1440	3660	39.3		9.1	7 days postoperatively
348A	April 26, 1938	F	38	149.5	49.8	1.42	3650		14	+47	2160	1900	4060	46.5		11.8	
348B	May 10 1938			149.5	46.9	1.37	3425			+28	1900	1280	3180	40.4		7.1	11 days postoperatively
350A	May 16, 1938	M	62	176.0	84.2	1.99	6000		16	+49	4330	2500	7130	39.3		18.9	
350C	June 11, 1938			176.0	82.2	1.98	5975		24	-2	4100	2380	6480	36.7		8.5	5 days postoperatively
351A	May 19 1938	F	55	148.8	59.6	1.53	3950		16	+60	2440	1610	4050	39.6		2.5	
351B	June 10 1938			148.8	56.0	1.48	3825		17	+26	1720	1470	3190	46.1		16.6	9 days postoperatively
352A	May 24, 1938	M	50	175.0	68.6	1.82	5400		12	+53	3250	2690	5940	45.3		10.0	
352B	June 22, 1938			175.0	69.4	1.83	5450		16	+6	3090	2170	5170	42.2		5.1	9 days postoperatively
356	June 6, 1938	F	35	160.0	55.6	1.59	4075		12	+46	2150	1580	3730	42.3		8.5	
7 PATIENTS WITH MYXEDEMA																	
10	December 11, 1934	F	53	165.5	72.1	1.78	4150	90	23	-15	3070	1170	4170	26.4		0.5	
29	May 18, 1935	F	62	161.5	63.6	1.66	4150	65	45	-17	2030	1530	3560	43.0		14.2	
234A	September 22, 1936	F	45	172.5	83.3	1.81	4100	95	26	-43	2490	1210	3700	32.7		9.8	
234B	October 13, 1936			172.5	84.3	1.63	4125	100	20	-20	2455	1235	3720	33.2		9.8	On thyroid medication
237	October 20, 1936	F	42	158.8	68.6	1.63	4150	60	23	-16	2120	1400	3520	39.8		15.2	
241C	December 11, 1936	M	39	176.5	76.0	1.91	5725	45	35	-21	2650	2020	4700	43.0		17.9	Post-total thyroidectomy
271D	April 1 1937	M	72	177.5	75.4	1.92	5775	90	22	-23	2730	2240	4970	45.0		13.9	Pericious anemia
353A	May 25, 1938	F	59	153.8	69.2	1.66	4150		22	-33	1850	1290	3150	40.8		24.1	
353B	July 7 1938			153.8	65.7	1.62	4125			-12	2450	1410	3860	36.5		8.9	On thyroid medication

In Figure 1 is shown the distribution of cases of hyperthyroidism and myxedema above and below normal total blood volume as predicted on the basis of surface area. It is evident that in comparison with the curve of distribution of total blood volumes in normals, the cases with hyperthyroidism form a group with the volumes definitely increased above normal, and that the cases

with myxedema form a group with definitely subnormal volumes. Thus in the 25 cases of hyperthyroidism 17 cases had a total blood volume which was within plus or minus 10 per cent of predicted normal value, 8 were above 10 per cent above normal, while none were less than 10 per cent below normal. In the group of patients with myxedema only one case had a volume in

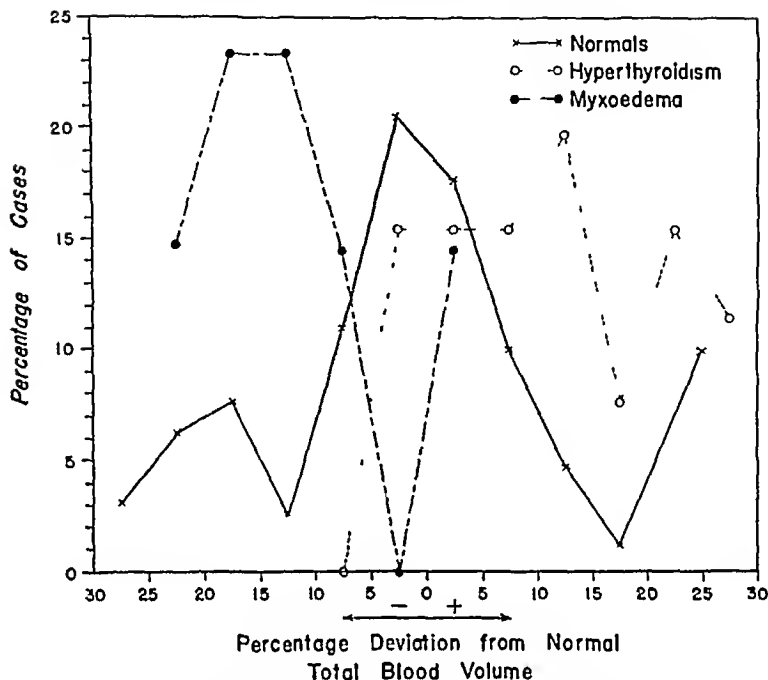


FIG. 1 THE DISTRIBUTION OF THE PERCENTAGE OF CASES ABOVE AND BELOW PREDICTED NORMAL TOTAL BLOOD VOLUME IN 25 CASES OF HYPERTHYROIDISM AND 7 CASES OF MYXEDEMA COMPARED TO THE DISTRIBUTION OF THE PERCENTAGE OF CASES ABOVE AND BELOW AVERAGE NORMAL TOTAL BLOOD VOLUME IN 99 NORMAL HUMANS

excess of predicted normal value, only 2 cases fell within limits of plus or minus 10 per cent of normal, and 5 cases had volumes more than 10 per cent below normal

The percentage deviation from predicted normal total blood volume based on surface area is shown in relation to basal metabolic rate in Figure 2. A direct relationship between deviation from predicted normal total blood volume and the levels of basal metabolic rate was observed. Prior to treatment, the circulation time was below normal in all except one case of hyperthyroidism and above normal in all the cases of myxoedema. Venous pressures were within the limits of normality of the method employed in all cases in which the determination was made. The direct relationship between the speed of blood flow and

basal metabolic rate reported by Blumgart, Gargill, and Gilligan (13) in hyperthyroidism was confirmed

No significant relationship between the percentage deviation from predicted normal volume based on surface area and circulation time or venous pressure was observed.

The change in the absolute total blood volume in 5 males and 10 females in relation to change in basal metabolic rate occurring during the course of therapy is shown in Figure 3. In all cases the basal metabolic rate was lower after operation, and in all except 1 case the total blood volume was less postoperatively than it was at the initial determination. Reduction in total blood volume postoperatively ranged from 540 to 770 cc., or an average of 650 cc. in males, and from 100 to 900

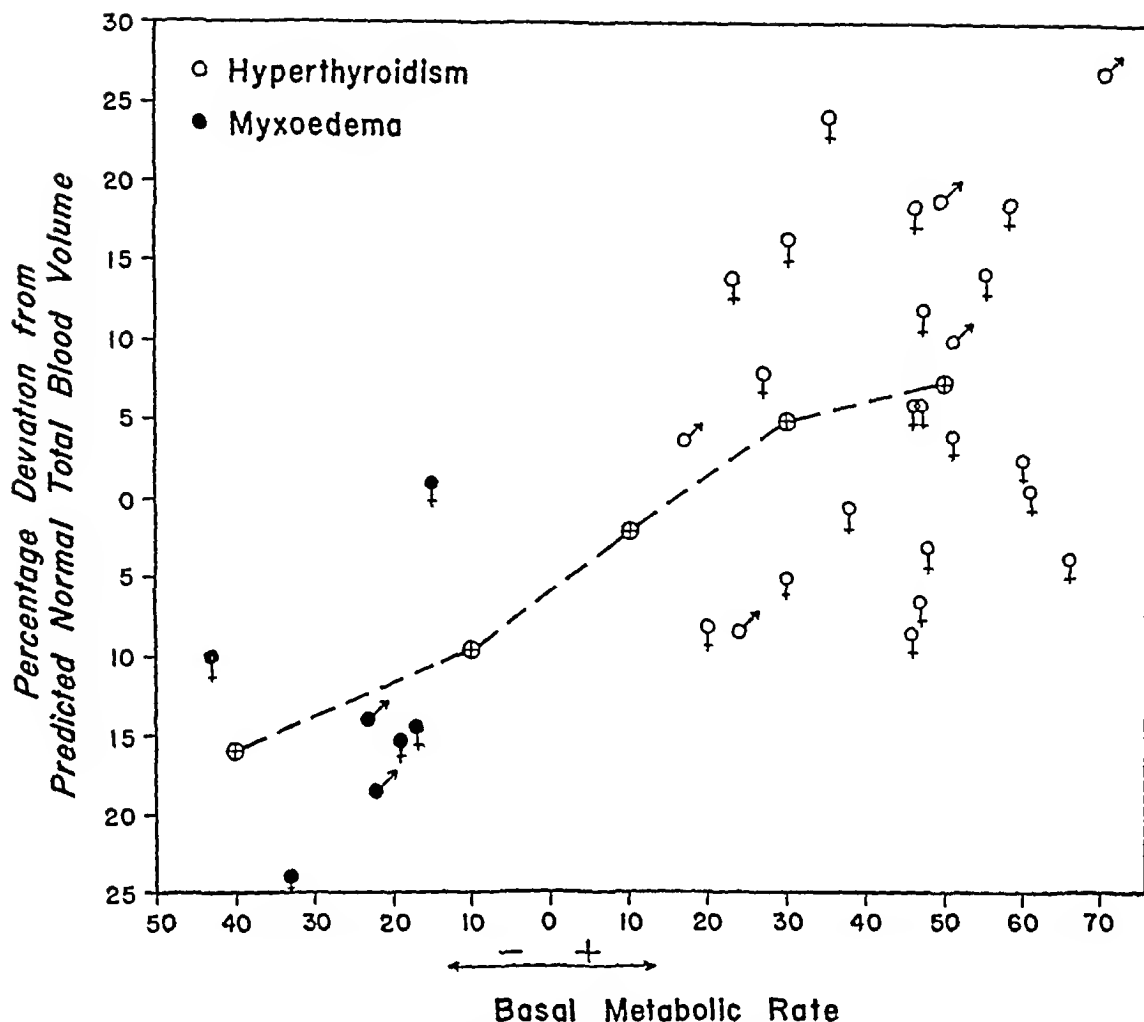


FIG 2 PERCENTAGE DEVIATION FROM NORMAL TOTAL BLOOD VOLUME AS PREDICTED ON THE BASIS OF SURFACE AREA IN 25 CASES OF HYPERTHYROIDISM AND 7 CASES OF MYXEDEMA IN RELATION TO BASAL METABOLIC RATE

cc. or an average of 500 cc. in females. In 1 male and 3 females the total blood volume decreased during the course of iodine administration. The total blood volume determined postoperatively was higher than that following iodine administration in 1 male and lower in 1 male and 1 female.

In 2 cases of myxedema, following the increase in basal metabolic rate resulting from thyroid therapy the total blood volume was unchanged in one and definitely increased in the other.

DISCUSSION

Our findings are somewhat at variance with those reported in the literature. Chang (4), using the CO method, found an average increase over his accepted normal value for total blood

volume of about 17 per cent in 21 cases of hyperthyroidism. Goldbloom and Libin (5), employing a modification of the dye method of Seydewitz and Lampe, found the total blood volume increased by 50 per cent over normal in 9 cases with hyperthyroidism. The average increase over normal in our series of 25 cases was only 6 per cent. Thompson (6) found the total blood volume to be about 25 per cent below normal in 9 cases of myxedema. The average in our group of 7 myxedematous patients was 15 per cent below normal.

In our opinion certain errors inherent in the earlier dye techniques, principally those arising from differences in the time required for the dye to become completely mixed in the blood stream

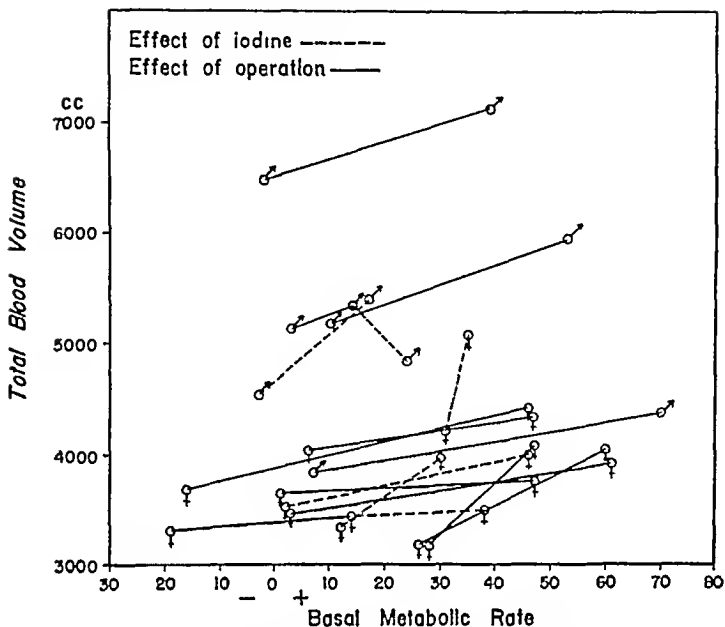


FIG. 3 DECREASE IN TOTAL BLOOD VOLUME IN HYPERTHYROIDISM WITH DECREASE IN BASAL METABOLIC RATE FOLLOWING THERAPY

Dotted lines indicate the change taking place under treatment with Lugol's solution only, solid lines indicate the change following subtotal thyroidectomy

in individuals with extremely rapid or slow circulation times (10) may be responsible for the discrepancies in results obtained by the above authors and those obtained in this study. The higher values found by Chang (4) may be attributed to the fact that the CO method probably measures myohemoglobin in addition to circulating oxyhemoglobin.

In both groups of patients in this series there were considerable variations in individual cases from the trend exhibited by the group as a whole, and therefore the determination of the blood volume has little, if any, practical value in the differential diagnosis of either hyperthyroidism or myxedema. Several factors account for these variations. In the determination of basal metabolic rates, oxygen consumption can be determined only within rather wide limits. It is common knowledge that errors are more prone to be

in the direction of too high rather than too low rates, and that the technical difficulties of accurate determinations increase with the severity of the clinical condition of the patient. In addition, as stated above, standards of normality are at best arbitrary values when applied to individuals and while the conclusions drawn from a sufficiently large group of cases may be valid, the findings in an individual case may not be in keeping with average trends.

Further evidence that in hyperthyroidism total blood volume determined at the height of the rise in metabolism is increased over the level of the individual in a normal metabolic state may be adduced from the fact that in all cases successful treatment of the disorder, as evidenced by a reduction in the metabolic rate, was accompanied by a prompt and considerable fall in the total blood volume. This reduction was greater than could

be accounted for by rest, or by the minor hemorrhage incident to operation. The reduction occurring coincident with the lowering of the metabolic rate was shared equally by plasma and red cell volume. The percentage of reduction in red cell volume occurring after therapy bears a linear relationship to the fall in metabolic rate as is shown in Figure 4.

In our opinion the total blood volume is definitely related to the oxygen requirement as expressed by the basal metabolic determination. Other than an increase above normal requirements in the total number of circulating red cells in hyperthyroidism and a decrease in myxedema, no explanation of the mechanism of the volume

change characteristic of these diseases has come to light.

None of these cases of hyperthyroidism had any evidence of valvular diseases or hypertensive heart disease nor did they exhibit any of the physical signs of congestive heart failure. The greatest increase in total blood volume above normal in this series was 28 per cent and the average about 6 per cent. In frank congestive failure the average increase in total blood volume above normal is 22 per cent (2). It would appear that even in the presence of an increased cardiac burden, the mechanical disadvantage imposed by the degree of hypervolemia experienced by these thyrotoxic patients was not enough to

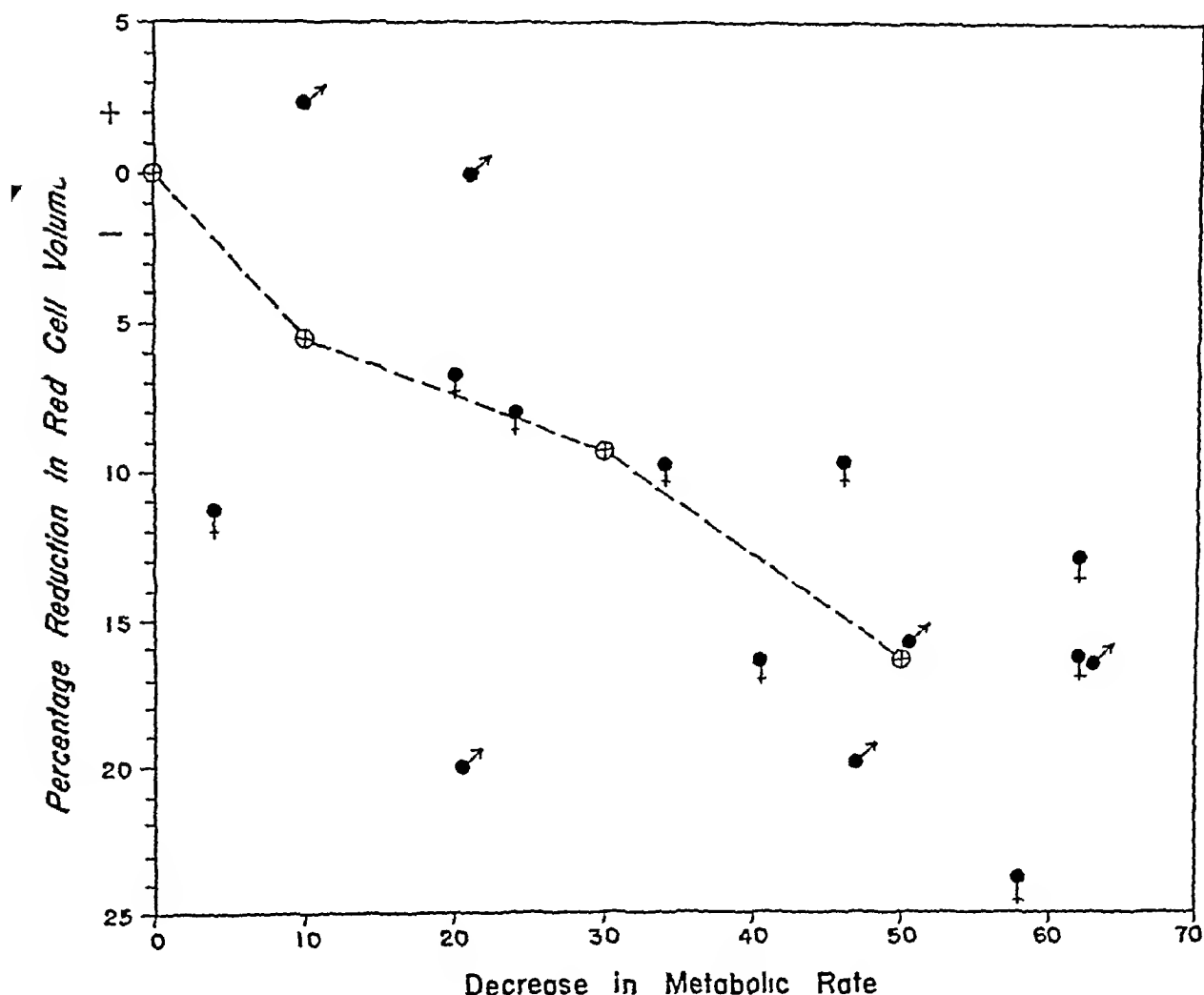


FIG. 4. PERCENTAGE REDUCTION FROM INITIAL CIRCULATING RED CELL VOLUME OCCURRING AFTER THERAPY IN HYPERTHYROIDISM IN RELATION TO THE ABSOLUTE DECREASE IN BASAL METABOLIC RATE.

precipitate congestive heart failure. However, in the absence of known specific pathological changes in the thyroid heart (14, 15), the hyperolemia brought about by the increased oxygen requirement of this diseased state may offer some explanation as to the mechanism of congestive heart failure in cases of hyperthyroidism.

CONCLUSIONS

(1) In 25 cases of hyperthyroidism the total blood volume was increased above normal on an average of 5.45 per cent, and 15.5 per cent below normal in 7 cases of myxedema.

(2) The deviation from normal in the untreated hyperthyroid state bears a linear relationship to the oxygen requirement as measured by the determination of the basal metabolic rate.

(3) In hyperthyroidism successful treatment is accompanied by a decrease in red cell and total blood volume commensurate with the lowering in basal metabolic rate.

We wish to express our thanks to Drs. H. L. Blumgart and M. D. Altschule of the Beth Israel Hospital Boston for permission to utilize clinical material. Miss Evelyn Berstein gave valuable technical assistance.

BIBLIOGRAPHY

- Gibson, J. G., 2d, and Evans, Wm. A., Jr., Clinical studies of the blood volume. II. The relation of plasma and total blood volume to venous pressure, blood velocity rate physical measurements, age and sex in ninety normal humans. *J. Clin. Invest.*, 1937, 16, 317.
- Gibson, J. G., 2d, and Evans, Wm. A., Jr., Clinical studies of the blood volume. III. Changes in blood volume, venous pressure and blood velocity rate in chronic congestive heart failure. *J. Clin. Invest.*, 1937, 16, 851.
- Rowntree, L. G., and Brown, G. E., *The Volume of the Blood and Plasma in Health and Disease*, W. B. Saunders Co., Philadelphia, 1929.
- Chang, H., The blood volume in hyperthyroidism. *J. Clin. Invest.*, 1931, 10, 475.
- Goldbloom, A. A., and Libin, I., Clinical studies in circulatory adjustments. I. Clinical evaluation of studies of circulating blood volume. *Arch. Int. Med.*, 1935, 55, 484.
- Thompson, W. O., Studies in blood volume. I. The blood volume in myxedema with a comparison of plasma volume changes in myxedema and cardiac edema. *J. Clin. Invest.*, 1926, 2, 477.
- Blumgart, H. L., Gargill, S. L., and Gilligan, D. R., Studies on velocity of blood flow. XIV. The circulation in myxedema with a comparison of the velocity of blood flow in myxedema and thyrotoxicosis. *J. Clin. Invest.*, 1931, 9, 91.
- Holbøll, S. A., Über die Grösse der blutmenge bei patienten mit myxodem. *Studien der blutmenge*, Acta med. Scandinav., 1930, 73, 538.
- Roth, P., Modifications of apparatus and improved technique adaptable to the Benedict type of respiration apparatus. *Boston M. and S. J.*, 1922, 186, 457.
- Gibson, J. G., 2d, and Evans, Wm. A., Jr., Clinical studies of the blood volume. I. Clinical application of a method employing the azo dye "Evans Blue" and the spectrophotometer. *J. Clin. Invest.*, 1937, 16, 301.
- Evans, Wm. A., Jr., Venous pressure. *New England J. Med.*, 1932, 207, 1934.
- Winternitz, M., Deutsch, J., and Brull, Z., Eine klinisch brauchbare Bestimmungsmethode der Blutumlaufzeit mittels Decholininjektion. *Med. Klin.*, 1931, 27, 986.
- Blumgart, H. L., Gargill, S. L., and Gilligan, D. R., Studies on velocity of blood flow. XIII. The circulatory response to thyrotoxicosis. *J. Clin. Invest.*, 1930, 9, 69.
- Rake, G., and McEachern, D., A study of the heart in hyperthyroidism. *Am. Heart J.*, 1932, 8, 19.
- Weller, C. V., Wanstrom, R. C., Gordon, H., and Bugher, J. C., Cardiac histopathology in thyroid disease. *Am. Heart J.*, 1932, 8, 8.

URINARY EXCRETION OF ANDROGENIC SUBSTANCES AFTER INTRAMUSCULAR AND ORAL ADMINISTRATION OF TESTOSTERONE PROPIONATE TO HUMANS¹

BY RALPH I. DORFMAN AND JAMES B. HAMILTON

(From the Adolescence Study Unit, the Laboratory of Physiological Chemistry and the Department of Anatomy, Yale University School of Medicine, New Haven)

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The urinary excretion of estrogens following administration of female sex hormones has been compared with the relief obtained from hot flushes and other phenomena accompanying the menopause (1) and has been studied with regard to the percentage of the administered material which appears in the urine (3 to 12 per cent) (2). Studies of the excretion of urinary androgens following the treatment of patients with testosterone propionate are reported below with regard to (a) the rapidity of the appearance and comparative levels of excretion in oral and intramuscular treatment, (b) the percentage of recovery in the urine of administered material, and (c) the evaluation of urinary levels of androgens as criteria of effective levels of male hormone in the body.

METHODS, MATERIALS, AND SUBJECTS

All urine samples were collected on a 24 hour basis and extracted with benzene in a continuous extractor according to the method previously described (3). The assays for androgenic activity contained in the urine extracts were performed on the day-old chicks comb (4). A minimum of 15 and usually 20 chicks were used for each assay. The accuracy of the determinations is considered to be ± 25 per cent and probably greater as can be calculated from previous data (4). All values are expressed as international units (IU) per 24 hours. The international unit of androgenic activity is that evoked by 0.1 mgm. of androsterone.

The androgen used was testosterone propionate.² In intramuscular administration employed a standard dose of 20 mgm. dissolved in 1 cc. of peanut oil since the amount and the type of oil influences the absorption (5). Injections were made intramuscularly into the buttocks or upper arms.

¹ This investigation was aided in part by grants from the Fluid Research Fund of Yale University School of Medicine, the General Education Board of the Rockefeller Foundation, and The International Cancer Research Foundation.

² Furnished through the courtesy of the Ciba Company under the trade name Perandren.

Crystalline testosterone propionate was given *per os* in disc shaped tablets 0.5 cm. in width 0.3 cm. in diameter. Tablets were taken on an empty stomach at spaced intervals during waking hours: $\frac{1}{2}$ hour before breakfast, 1 hour before lunch, 1 hour before dinner, and in the evening before retiring.

The subjects are 2 men with organic and functional evidence of deficient testicular secretion. Case 1 is a 35 year-old man who had lived until the age of 35 with a single descended testis. He has a wife and 2 children. Following surgical removal of the testis at the age of 35 castration phenomena (6) including the following appeared: atrophy of the genitalia, muscular weakness and fatigue (7), absence of penile erection (8, 9), presence of vasomotor phenomena such as may be found in women at the menopause, skin pallor, and inability to tan (10). Before androgenic administration, the level of output of male hormone in 4 urine assays covering 7 days taken from a period of weeks showed a daily average of 13.5 IU. This is far below the levels of androgen excretion of normal adult men (11, 12, 3). Still further evidence that if any abdominal testis were present it did not secrete large amounts of androgens was the negative clinical response obtained with 16 injections of large amounts of anterior pituitary like substance, 100 rat units (R.U.) given three times weekly.

Case 2 is a 27 year-old sexually underdeveloped man, described in detail elsewhere (8) who exhibited a like condition save that the genitalia instead of undergoing atrophy had never developed. The testes did not respond to injections of 100 R.U. of anterior pituitary like material given 3 times weekly for 9 weeks.

RESULTS

A. Intramuscular administration. Intramuscular injections of 20 mgm. of testosterone propionate were given daily to Case 1 for 30 successive days. The resultant increased excretion of urinary androgens is shown in Table I. The average daily output prior to injections was 13.5 IU. By the 3d day of injection, the output was 50 IU at which time some of the clinical symptoms of androgen deficiency remained, particularly the vasomotor phenomena. This is perhaps correlated with the fact that the longer the period

TABLE I

*Urinary androgenic activity before, during, and after intramuscular injections of testosterone propionate**

Day of treatment	Day after cessation of treatment	Urinary androgenic activity <i>I U per day</i>
0		7
0		21
3		50
4		93
5, 6		47
9, 10		78
12, 13, 25, 26, 27		68
30		94
	8, 9	38
	30	13
	37	13

* Intramuscular injections of 20 mgm of testosterone propionate (equivalent to 1110 I U) in 1 cc of peanut oil were given Case 1 daily for 30 successive days. The urinary output of comb growth-stimulating materials reached the levels of normal men the 3d day of injection and remained so during the 30 days of treatment. Thirty days after the end of injections, the amount in the urine was again down to a pre-injection level. Castration signs disappeared during this treatment.

of androgen injection the greater the suppression of pituitary hyperactivity (13)

This high level of androgen excretion was maintained throughout the period of treatment, as shown by assays of 11 out of the 16 remaining days of injection. The average daily output of 12 days, ranging from the 3d to the 30th day was 68.9 I U, an increase of 510 per cent. In all determinations this level remained within the range of 40 to 100 I U which is the range for normal men as determined by several groups of workers (11, 12, 3). After the first few days castration symptoms remained absent throughout the course of treatment. Thus, with the intramuscular injections which maintained normal ranges of urinary androgens, the patient remained symptom-free.

Eight to 9 days after the cessation of injections, the daily output was still 38 I U. Although this was an increase of 281 per cent over the castrate level and can hardly be considered lower than normal levels, castration signs were again of considerable severity.

Determinations done 30 and 37 days respectively after the end of treatment showed 13 I U, a return to low pre-injection levels.

B Oral administration Daily oral administration of 60 mgm of testosterone propionate was given to Case 1. An immediate high level of urinary androgen was obtained. A 24-hour specimen between the 36th and 60th hour after ingestion of the first tablet contained 137 I U, an increase of 124.5 I U over the pre-treatment level of daily excretion (Table II). This increase of

TABLE II

*Urinary androgenic activity before and during oral administration**

Case	Day of treatment	Urinary androgenic activity <i>I U per day</i>
1	0	7
	0	21
	0	13
	0	13
	3	137
	22	500
2	0	14
	0	18
	2	38
	7, 9, 11, 12	94
	14	264

* Oral administration of 60 mgm of testosterone propionate (equivalent to 3330 I U) in tablet form was given Case 1 daily for 15 days followed by 120 mgm (equivalent to 6660 I U) daily for the next 7 days. Case 2 received 60 mgm of testosterone propionate daily for 14 days. The urinary androgenic activity far exceeded the levels for normal men, but the patients were only slightly relieved of castration phenomena.

922 per cent in urinary androgen activity was accompanied by slight, if any, clinical improvement. The amount ingested was then raised to 120 mgm of testosterone propionate daily and after 3 days the daily androgen excretion was 500 I U, an increase of 3703 per cent over the pre-treatment level. Castration symptoms still continued.

Case 2 had an average urinary output of 16 I U before oral administration was begun. By the 2d day of daily oral administration of 60 mgm of testosterone propionate, 38 I U were excreted in a 24-hour specimen of urine (Table II). Pooled samples for the 7th, 9th, 11th and 12th days of treatment gave an average of 94 I U. By the 14th day 264 I U were excreted, an increase of 1560 per cent over the level before treatment. A moderate amount of clinical relief occurred but it was not equal to that obtained

with 1/6 of this dose given subcutaneously in injections of 20 mgm 3 times weekly

If the samples assayed could be considered representative, a very rough estimate in Case 1 of the percentage of recovery in the urine of comb growth stimulating material with daily subcutaneous injections might be made as in Table III

TABLE III

Percentage of administered androgenic material recovered in the urine

	Average urinary androgen in 24 hours	Total
	I U	I U
Days 1 to 10 of treatment	67.00	670
Days 11 to 30 of treatment	72.33	1446
Days 1 to 15 after treatment	38.00	570
Total excretion		2686
Total excretion expected over these 45 days without treatment (at 13.5 I U daily)		607
Net excretion		2079

The 600 mgm. of administered testosterone propionate is equivalent to 502 mgm. of testosterone
If urinary androgen is testosterone

Since 1 I U of testosterone = 0.015 mgm. (cf Koch (17))
then 2079 I U (net excretion) = 31.190 mgm

Percentage recovery = $\frac{31.19 \text{ mgm.}}{509 \text{ mgm.}} \times 100 = 6.2 \text{ per cent}$

If urinary androgen is androsterone:

Since 1 I U of androsterone = 0.100 mgm
then 2079 I U (net excretion) = 207.900 mgm

Percentage recovery = $\frac{207.9 \text{ mgm.}}{502 \text{ mgm.}} \times 100 = 41.4 \text{ per cent}$

If urinary androgen is dehydroisoandrosterone

Since 1 I U of dehydroisoandrosterone = 0.300 mgm
then 2079 I U (net excretion) = 623.700 mgm

Percentage recovery = $\frac{623.7 \text{ mgm.}}{502 \text{ mgm.}} \times 100 = 124.2 \text{ per cent}$

If urinary androgens are an equal mixture of dehydroisoandrosterone and androsterone

Percentage recovery = $\frac{313.5 \text{ mgm.}}{502 \text{ mgm.}} \times 100 = 62.4 \text{ per cent}$

If the androgenically active material is excreted in the form of testosterone, the percentage of recovery is 6.2. If it is excreted as androsterone the recovery is 41.4 per cent, as dehydroisoandrosterone 124.0 per cent and as equal mixtures of androsterone and dehydroisoandrosterone 62.4 per cent

The possibility that all of the testosterone propionate is converted into dehydroisoandrosterone

is unlikely since, as shown in Table III, this would mean an excretion of more material than was administered.

There is no proof that the pre treatment level of male hormone secretion by the body was maintained during the injection of the testosterone propionate, especially since this intramuscular administration of androgen greatly decreased the urinary level of gonadotropic (follicle stimulating) substance in this patient (14). If none of the excreted androgen were of endogenous origin, more of the amount excreted would be considered as recovered from the administered hormone. This would make estimates of the amount recovered as much as $\frac{1}{2}$ higher

DISCUSSION

Even if the figures for recovered hormone are no higher than listed, there remain the obvious facts that (1) daily intramuscular injections of 20 mgm of testosterone propionate maintained excretion levels at approximately those determined for normal men and that castration symptoms disappeared during this treatment, and (2) orally administered crystalline testosterone propionate was readily absorbed from the gastro-intestinal tract and eliminated in large amounts through the kidneys, since, however, no satisfactory clinical relief was afforded even when absorption was continued during 16 hours of the day, it seems likely that the material was eliminated with great rapidity. The figures indicate that increasing the oral dose of crystalline testosterone propionate can be expected to result in only exceedingly brief levels of hormone in the fluids and tissues of the body. In the maintenance of effective levels in the body by oral means there must be a balance between slow absorption to avoid prompt elimination by the kidneys and ready absorption to prevent waste in the feces. Furthermore, damage (15, 16) from large doses of male hormone may be incurred, especially in the kidney and the vascular system.

The transient presence of orally administered testosterone propionate within the body and the rapid appearance of large quantities of androgenic material in the urine raise the question of threshold levels. It may be that when a certain threshold is reached, the hormone above this level is

excreted in the urine. In this connection it is interesting to note that in adrenal virilism in women, enormous titers of androgen activity in the urine may be observed, but the masculinization does not proceed beyond that of the normal male.

The urinary level of androgen is an accurate reflection of the level in the tissues and fluids of the body only when the supply in circulation is at a maintained rate. Temporary presence of a large amount in the body would produce a large urinary excretion. In other words, evaluation of levels of androgens in the body by comparison of one urine titer with another would be misleading if the androgen precursors were not present over the same general length of time. Further, measurements of urinary androgenic activity should be expected to account only with inaccuracy if there were great variations in the amount present in the body during this time.

If the androgens secreted by the body were from the testes and in the form of testosterone and had a percentage of recovery in the urine like that from intramuscular injections in Case 1, there would be secreted in 25 gram testes of a man, 22 IU per gram every 24 hours.

SUMMARY

1 In the patient with low levels of testicular secretion both intramuscular and oral administration of testosterone propionate produce an increase in the androgenic activity of the urine.

2 With intramuscular injections of 20 mgm daily, Case 1 showed an increase in output to normal levels and clinically a disappearance of castration phenomena. A 24-hour titer of 50 IU was obtained on the 3d day of administration. The average of 6 assays covering 12 of the 30 days of injection was 68.9 IU, with a range from 47 to 94 IU.

3 Tablets *per os* of 60 to 120 mgm daily gave 24-hour urine readings as high as 500 IU in Case 1, and 264 IU in Case 2. These large excretions were not accompanied by as good clinical relief as obtained with $\frac{1}{6}$ to $\frac{1}{4}$ the amount taken intramuscularly and with lesser androgenic activity of the urine.

4 Absorption of the large amounts of androgen can take place through the gastro-intestinal tract

with what appears to be rapid elimination through the kidneys. It is suggested that there may be a threshold for the substance in the body and that rapid disposition is made of an excess. Oral means of administration should be considered from the standpoint of material lost not only through the feces but also excreted in the urine.

5 Urine assays as an indication of the presence of hormone in the body may be misleading if the hormone in the body is present only irregularly. For example, enormous quantities of urinary androgen were found with an oral method that appeared to give only transient levels in the body.

6 A rough estimation of the percentage of the androgenic material recovered in the urine is 62 per cent, if it be in the form of testosterone, 41.4 per cent if androsterone, 62.4 per cent if an equal mixture of androsterone and dehydroisandrosterone.

BIBLIOGRAPHY

- 1 Mazer, C and Isreal, S L., Studies on the optimal dosage of estrogens, an experimental and clinical evaluation. *J A M A*, 1937, 108, 163.
- 2 Kemp, T and Pedersen-Bjergaard, K., Absorption and excretion of oestrone by the human organism. *Lancet*, 1937, 2, 842.
- 3 Gallagher, T F, Peterson, D H, Dorfman, R I, Kenyon, A T and Koch, F C, The daily urinary excretion of estrogenic and androgenic substances by normal men and women. *J Clin. Invest.*, 1937, 16, 695.
- 4 Dorfman, R I and Greulich, W W, The response of the chick's comb to naturally occurring androgens and estrogens. *Yale J Biol and Med*, 1937, 10, 80.
- 5 Parkes, A S, Increasing the effectiveness of testosterone. *Lancet*, 1936, 2, 674.
- 6 Hamilton, J B and Hubert, G, Unpublished data.
- 7 Miller, N E, Hubert, G and Hamilton, J B, Mental and behavioral changes following male hormone treatment of adult castration, hypogonadism and psychic impotence. *Proc. Soc. Exper Biol and Med.*, 1938, 38, 538.
- 8 Hamilton, J B, Treatment of sexual underdevelopment with synthetic male hormone substance. *Endocrinology*, 1937, 21, 649.
- 9 Hamilton, J B., Induction of penile erection by male hormone substances. *Endocrinology*, 1937, 21, 744.
- 10 Hamilton, J B and Hubert, G, Photographic nature of tanning of the human skin as shown by studies of male hormone therapy. *Science*, 1938, 88, 481.
- 11 Dingemans, E., Borchardt, H and Laqueur, E., Capon comb growth-promoting substances ("male

- hormones') in human urine of males and females of varying age. *Biochem. J.*, 1937, 36, 500
12. Callow R. K. Extraction of male hormone from urine for biological assay. *Lancet*, 1936, 2, 565
13. Hamilton J. B. and Wolfe, J. M., The effect of synthetic androgen upon the gonadotropic potency of the anterior pituitary. *Endocrinology* 1938, 22, 360
14. Catchpole, H. and Hamilton, J. B., Unpublished data.
15. Hamilton, J. B., Precocious masculine behavior following administration of synthetic male hormone substance. *Endocrinology* 1938, 23, 53
16. Hamilton J. B. and Dorfman, R. L., Influence of various solvents upon the length and strength of the action of synthetic male hormone testosterone propionate. (To be published.)
17. Koch F. C., The male sex hormones. *Physiol. Rev.*, 1937, 17, 180

THE RATE OF FILTRATION THROUGH THE CAPILLARY WALLS AS MEASURED BY THE PRESSURE PLETHYSMOGRAPH OBSERVATIONS ON CONTROL SUBJECTS AND ON PATIENTS WITH INTRAHEPATIC DISEASE, THYROTOXICOSIS, AND MYXEDEMA

By BENJAMIN V. WHITE¹ AND CHESTER M. JONES

(From the Medical Clinic of the Massachusetts General Hospital and the Department of Medicine of the Harvard Medical School Boston)

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The frequent occurrence of telangiectasis ecchymoses, bleeding tendencies, ascites, and peripheral edema in the various types of intrahepatic disease suggested the value of a study of the permeability of the capillaries in such subjects. The traumatic methods of estimating capillary permeability (1, 2, 3) appeared to show a too wide range of normal values. The dye injection method (4) was not available for human use. The use of the plethysmograph was therefore elected. It was desired to obtain evidence, if possible, as to whether peripheral edema in particular, in serious hepatic disease, resulted in part from increased capillary permeability.

Landis and Gibbon (5) modified the pressure plethysmograph of Krogh, Landis, and Turner (6) so as to eliminate errors caused by differences in the degree of contraction of blood vessels during the volumetric readings. This they accomplished by exerting a pressure of 200 mm Hg upon the fluid in the plethysmograph during the reading periods. Such a pressure was adequate to compress the entire vascular tree and to record with a moderate degree of accuracy small changes in the amount of interstitial fluid. Readings of reduced arm volume (under pressure of 200 mm Hg) were made before and after obstruction to the venous outflow from the arm, and the rate of fluid filtered was calculated from the increase in reduced arm volume and the volume of the arm which had actually been in the plethysmograph.

Landis and Gibbon's studies were confined to two normal male subjects so that the filtration rate was not complicated by depleted serum protein and low colloid osmotic pressure. Smirk (7) also approached the problem of capillary permeability by the measurement of changes in arm volume before and after venous obstruction. He

made determinations in patients with the nephrosis syndrome and with hepatic insufficiency, in both of which conditions the serum proteins were depleted. Hence the pressure in the cuff which occluded the venous outflow was not set at an arbitrary absolute figure but was regulated so as to be greater by a constant amount than the colloid osmotic pressure of the serum. The colloid osmotic pressure was measured directly by the use of the osmometer of Verney (8).

Starling (9) showed that the colloid osmotic pressure of the human serum was roughly proportional to the concentration of protein which it contained. Govaerts (10) subsequently concluded that one gram per cent of serum albumin exerted a colloid osmotic pressure of 7.54 cm H₂O while one gram per cent of serum globulin exerted a colloid osmotic pressure of 1.95 cm H₂O. Although some justified criticism of these figures has appeared, Wies and Peters (11) made an empirical study of human sera which tended to confirm the validity of estimates of colloid osmotic pressure based on the concentrations of different protein fractions. Wies and Peters found that estimates based upon the total protein alone were less accurate than those based upon albumin and globulin separately. They found, however, that estimates of colloid osmotic pressure based upon specific factors for albumin and globulin were inclined to be low for protein levels in the higher range of normal. Hence they prepared a formula from which they found that more accurate estimations could be made. (See Method.)

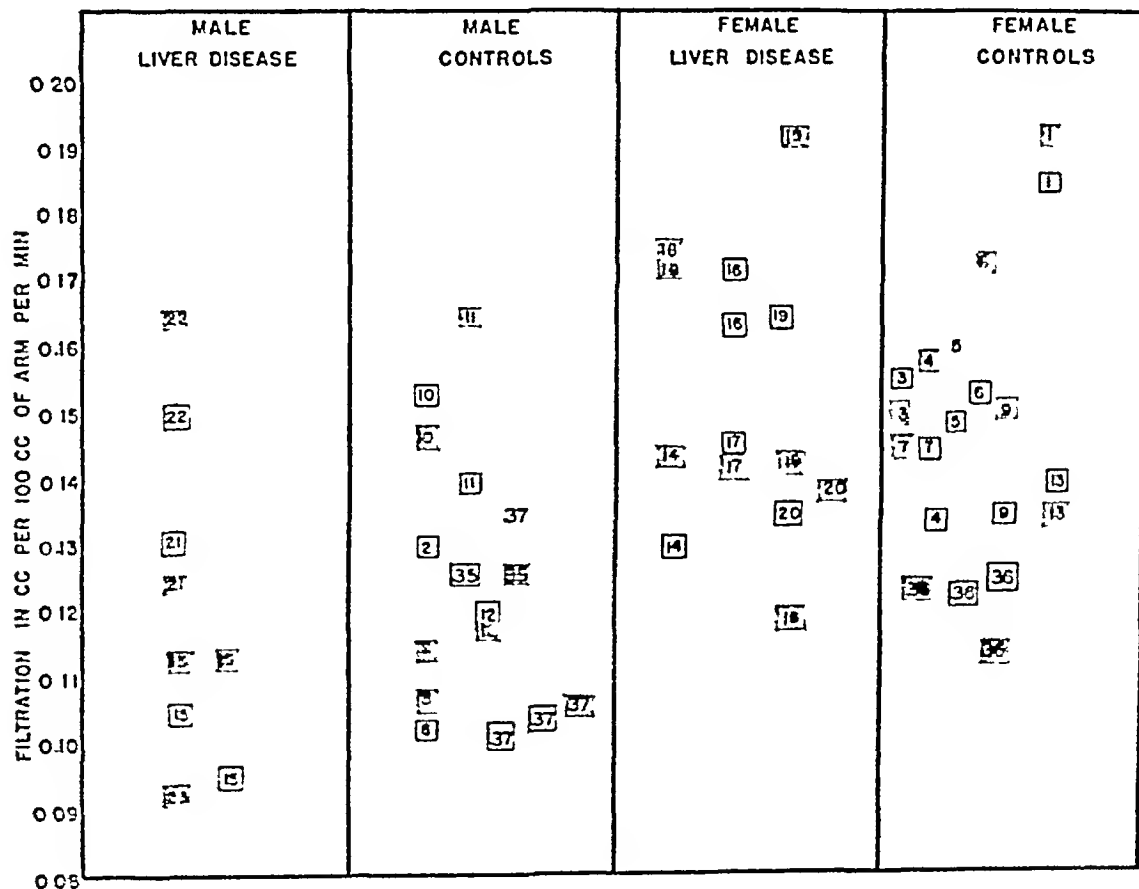
In this study a combination of the methods of Landis and Gibbon and of Smirk was employed. The pressure plethysmograph was used, but the obstructing venous pressure was in every case greater by a constant amount than the colloid

¹Jeffrey Richardson Fellow—Harvard Medical School.

osmotic pressure of the serum. The colloid osmotic pressure of the serum was estimated from the concentrations of serum albumin and serum globulin by the use of the formula of Wies and Peters to be described below.

Studies were made on a group of normal controls largely recruited from young doctors, technicians, and secretaries about the hospital, and on a group of patients with severe liver disease. This latter group included patients with severe secondary biliary cirrhosis, some of whom were observed both before and after surgical relief of the obstruction, patients with toxic cirrhosis (Laennec's cirrhosis), and one patient with subacute yellow atrophy. Many of these persons had frank evidence of peripheral edema, ascites, ecchymoses, telangiectasis or bleeding tendencies.

Observations were also made on a series of eight patients with frank hyperthyroidism, at the time of admission to the hospital, after the effect of iodine therapy, and one month or more post-operatively. A series of three patients with myxedema was studied both before and after thyroid therapy. Isolated observations were made on one patient with acute nephritis with edema (Subject 35), on one patient with urticaria (Subject 36) during a menstrual period and between menstrual periods, and on one patient with peptic ulcer (Subject 37). This last patient was studied twice, once when his blood vitamin C level was 0.1 mgm per cent and subsequently when it had been elevated by intravenous therapy to 0.7 mgm per cent. These three patients are included with the control group in Figure 1.



METHOD

The operation of the pressure plethysmograph has been adequately described by Landis and Gibbon (5).

Repeated determinations of the reduced arm volume were made at 10 minute intervals until successive determinations checked within 1 cm. A cuff which had been placed upon the upper arm was then inflated for a period of 10 minutes at a pressure which would raise the hydrostatic pressure of the veins within the plethysmograph to a point exactly 20 cm. H_2O above the colloid osmotic pressure of the serum. The method of calculating the cuff pressure is described below.

After the 10-minute period of venous congestion, a final determination of reduced arm volume was made. The difference between this final determination and the last determination previous to inflation of the cuff represented the actual amount of fluid which had filtered through the capillary walls. If divided by the number of cubic centimeters of arm volume in the plethysmograph, it represented the fraction of a cubic centimeter filtered per cubic centimeter of arm in 10 minutes. To conform with the system of recording used by Landis and Gibbon, the decimal point was moved one space to the right so as to represent the amount of fluid filtered per 100 cc. of arm in one minute. This figure was used throughout the illustrations.

With two exceptions all readings were checked by successive determinations on both arms. In most instances the readings of the two arms were moderately close, considering the sources of error imposed by the technique. In several instances the two arms did not check. In those cases the arm which was presumed to be wrong was restudied subsequently. If it then checked with the expected reading, no further study was made. If it failed to check, the first arm was also repeated. In the case of Patient 29 with thyrotoxicosis there was a wide discrepancy between the readings of the two arms. In this instance, before operation, the left arm was studied three times and the right arm twice. The checks in this case were moderately close and always showed the left arm to have a much lower rate than the right. The rates of the two arms were, however usually moderately close and served as a check on the technique.

The methods followed in this study are identical with those of Landis and Gibbon except in the following details.

1 Temperature Observations were made in a room which was maintained at almost constant temperature throughout each determination. Fluctuations of more than 1 C. were unusual. Landis and Gibbon found that fluctuations in room temperature affected their results but little. The plethysmograph temperature was maintained between 34.5° and 35.5 C. throughout all readings. Landis and Gibbon found the rate of filtration to vary proportionately with the temperature of the plethysmograph, the filtration rate at 45 C. being roughly twice that observed at 15 C. For the purposes of this study 35 C. was chosen because it was easy to maintain and was most comfortable for the patient.

2 Pressure in the venous cuff Inasmuch as the fore arm was in a vertical position, the cuff about the brachium was lower than the plethysmograph by a considerable distance. In order to maintain the desired hydrostatic pressure within the veins of the forearm it was necessary to add to the pressure within the cuff enough additional pressure to offset the elevation of the plethysmograph. Landis and Gibbon added a number of centimeters of water equal to the distance from the core of the brachium to the top of the plethysmograph. For convenience the same points of reference were used in this study. The number of centimeters added for this purpose varied from 19 to 22. In Tables I, II and III this hydrostatic factor is omitted and only the effective pressure within the plethysmograph is recorded.

In order to produce filtration Smirk (7) added 20 cm. H_2O to the colloid osmotic pressure of the serum. Since the arm was in the horizontal position the hydrostatic factor was not added. In the present study the hydrostatic factor (usually 20 to 22) was added to the constant factor (20) and the sum of these two was added to the colloid osmotic pressure of the serum.

The colloid osmotic pressure of the serum was calculated from the fifth formula of Wies and Peters

$$COP = A/W \times 60.9 + G/W \times 22.9 - 50$$

where A/W equals the concentration of albumin in the water of the serum in grams per liter and G/W equals

TABLE I
Experimental data on control subjects

The effective venous pressure in each instance was 20 cm. above the colloid osmotic pressure of the serum. The cuff pressure actually applied because of the vertical position of the plethysmograph, exceeded this figure by 19 to 24 cm. H_2O .

Subject number	Colloid osmotic pressure of serum	Effective venous pressure	Volume of left arm	Increase in left reduced arm volume	Filtration rate in a. cm. per 100 cc. of arm volume per minute. Left arm	Volume of right arm	Increase in right reduced arm volume	Filtration rate, Right arm	Diagnosis
	H_2O	H_2O	c. cm.	c. cm.		c. cm.	c. cm.		
1	28.0	48.0	350	10.2	0.292	510	10.4	0.202	Normal
2	40.3	60.3	970	8.7	0.280	990	7.9	0.118	Normal
3	37.4	57.4	470	2.8	0.146	510	7.5	0.180	Normal
4	30.7	50.7	380	8.3	0.254	380	6.0	0.148	Normal
5	33.1	53.1	378	5.8	0.249	375	8.0	0.209	Normal
6	25.3	45.3	438	6.6	0.243	460	8.0	0.173	Normal
7	29.4	49.4	400	5.8	0.245	430	6.1	0.144	Normal
8	33.6	53.6	580	8.9	0.258	600	6.8	0.107	Normal
9	25.3	45.3	430	5.8	0.255	475	7.1	0.160	Normal
10	34.4	54.4	492	6.7	0.243	533	9.3	0.147	Normal
11	33.3	53.3	678	8.3	0.240	600	11.0	0.180	Normal
12	31.0	51.0	665	8.0	0.240	650	8.0	0.118	Normal
13	36.2	56.2	425	8.0	0.240	460	6.3	0.133	Normal
23	37.7	57.7	605	7.8	0.255	595	7.5	0.123	Neuritis
25	34.8	54.8	606	6.3	0.243	610	6.3	0.123	Driscoll's
26	34.8	54.8	603	6.3	0.243	610	6.8	0.114	Urticaria during menstrual period
27	36.3	56.3	635	6.9	0.210	635	8.5	0.164	Vitamin O in blood 0.1 mgm. per cent
27	36.3	56.3	635	6.5	0.104	635	6.7	0.104	Vitamin O in blood 0.7 mgm. per cent

TABLE II

Experimental data on patients with mixed edema

The readings are essentially similar to the control group

Patient number	Colloid osmotic pressure of serum	Effective venous pressure	Volume of left arm	Increase in left arm volume	Fluorimetric rate in cc. per 100 cc. of arm volume per minute	Volume of right arm	Increase in right arm volume	Fluorimetric rate in cc. per 100 cc. of arm volume per minute	Hydrostatic pressure of arm veins	Hydrostatic pressure of right arm	Diagnosis
14	31.7	1.7	47.3	6.7	0.113	55.3	7.5	0.114	6.5	6.5	Cardiac (ed.)
15	31.4	1.4	47.4	7.4	0.113	54.7	6.1	0.113	6.1	6.1	Cardiac (ed.)
16	31.4	1.4	47.4	6.4	0.113	55.0	5.9	0.113	6.0	6.0	Cardiac (postop)
17	31.4	1.4	47.4	6.4	0.113	55.0	5.9	0.113	6.0	6.0	Cardiac (ed.)
18	31.4	1.4	47.4	6.4	0.113	55.0	5.9	0.113	6.0	6.0	Cardiac (postop)
19	31.4	1.4	47.4	6.4	0.113	55.0	5.9	0.113	6.0	6.0	Cardiac (postop)
20	31.4	1.4	47.4	6.4	0.113	55.0	5.9	0.113	6.0	6.0	Cardiac (postop)
21	31.4	1.4	47.4	6.4	0.113	55.0	5.9	0.113	6.0	6.0	Cardiac (postop)
22	31.4	1.4	47.4	6.4	0.113	55.0	5.9	0.113	6.0	6.0	Cardiac (postop)
23	31.4	1.4	47.4	6.4	0.113	55.0	5.9	0.113	6.0	6.0	Cardiac (postop)
24	31.4	1.4	47.4	6.4	0.113	55.0	5.9	0.113	6.0	6.0	Cardiac (postop)

the concentration of globulin in the water of the serum in grams per liter, the colloid osmotic pressure being expressed in millimeters of water H' was calculated from the formula of Eisenmann, MacKenzie, and Peters (12)

$$H = 68.40 - 718 \times \text{Serum total protein.}$$

3 Preparation. The determinations were ordinarily made in the afternoon, commencing about one and one-half hours after the noon meal. Ordinarily the left arm was measured first and the right arm one and one-half hours later. After therapy the left arms of Patients 32, 33 and 34 were studied in the fasting state, as were the left arms of certain of the postoperative patients with thyrotoxicosis. Because of the possible significance of

TABLE III

Experimental data on patients with thyrotoxicosis and on patients with mixed edema

No effort although the b lateral readings usually checked moderately closely, there were great fluctuations in the rate from one therapeutic stage to another

Patient number	Colloid osmotic pressure of serum	Effective venous pressure	Volume of left arm	Increase in left arm volume	Fluorimetric rate in cc. per 100 cc. of arm volume per minute	Volume of right arm	Increase in right arm volume	Fluorimetric rate in cc. per 100 cc. of arm volume per minute	Hydrostatic pressure of arm veins	Hydrostatic pressure of right arm	Diagnosis
25	31.5	1.5	51.0	8.8	0.157	57.0	9.3	0.163	7.0	7.0	Thyrotoxicosis
26	31.5	1.5	51.0	8.8	0.157	57.0	9.3	0.163	7.0	7.0	Thyrotoxicosis
27	31.5	1.5	51.0	8.8	0.157	57.0	9.3	0.163	7.0	7.0	Thyrotoxicosis
28	31.5	1.5	51.0	8.8	0.157	57.0	9.3	0.163	7.0	7.0	Thyrotoxicosis
29	31.5	1.5	51.0	8.8	0.157	57.0	9.3	0.163	7.0	7.0	Thyrotoxicosis
30	31.5	1.5	51.0	8.8	0.157	57.0	9.3	0.163	7.0	7.0	Thyrotoxicosis
31	31.5	1.5	51.0	8.8	0.157	57.0	9.3	0.163	7.0	7.0	Thyrotoxicosis
32	31.5	1.5	51.0	8.8	0.157	57.0	9.3	0.163	7.0	7.0	Thyrotoxicosis
33	31.5	1.5	51.0	8.8	0.157	57.0	9.3	0.163	7.0	7.0	Thyrotoxicosis
34	31.5	1.5	51.0	8.8	0.157	57.0	9.3	0.163	7.0	7.0	Thyrotoxicosis

PATIENTS WITH THYROTOXICOSIS

25	31.5	51.0	8.8	0.157	57.0	9.3	0.163	+35 (+41) preop
26	31.5	51.0	8.8	0.157	57.0	9.3	0.163	+46 (+57) after iodine
27	31.5	51.0	8.8	0.157	57.0	9.3	0.163	-13 (-9) postop
28	31.5	51.0	8.8	0.157	57.0	9.3	0.163	+17 preop
29	31.5	51.0	8.8	0.157	57.0	9.3	0.163	+17 postop
30	31.5	51.0	8.8	0.157	57.0	9.3	0.163	+17 preop
31	31.5	51.0	8.8	0.157	57.0	9.3	0.163	+17 postop
32	31.5	51.0	8.8	0.157	57.0	9.3	0.163	+17 preop
33	31.5	51.0	8.8	0.157	57.0	9.3	0.163	+17 postop
34	31.5	51.0	8.8	0.157	57.0	9.3	0.163	+17 preop

PATIENTS WITH MIXEDEMA

32	31.7	55.7	7.7	0.117	7.0	7.4	0.113	-20
33	31.7	55.7	7.7	0.117	7.0	7.4	0.113	+8 after treatment
34	31.7	55.7	7.7	0.117	7.0	7.4	0.113	-20
35	31.7	55.7	7.7	0.117	7.0	7.4	0.113	+13 after treatment
36	31.7	55.7	7.7	0.117	7.0	7.4	0.113	-31
37	31.7	55.7	7.7	0.117	7.0	7.4	0.113	-11 after treatment

within the plethysmograph were identical for the two arms. However, the volume of each arm and the increase in reduced arm volume of each arm are recorded separately, so that the accuracy of the checks from one arm to the other may readily be observed. The final filtration rates are recorded separately for each arm both in Tables I, II, and III and in Figures 1 and 2 where they are represented in purely graphic form.

In Figure 1 are recorded the observations upon the control group and the group of patients with liver disease. Included in the control group are three patients with non hepatic diseases. The diagnoses of the patients suffering from diseases of the liver are noted in Table II. Both groups were partitioned on the basis of sex and this differentiation is shown in Figure 1 from a study of which it is apparent that the observed rate

of filtration was higher in the females than in the males. The limits of normal observed in the control groups were not exceeded in the liver disease groups. In fact, the upper and lower limits observed in female controls and female patients with liver disease were almost identical, as were those observed in the male groups.

At 35° C the rates in all subjects varied from 0.092 to 0.200 cc. per 100 cc. of arm volume in 1 minute. In two healthy adults (males) at a plethysmograph temperature between 34.0° and 35.0° C and at an effective venous pressure of 50 cm H₂O, Landis and Gibbon found the filtration rate to vary from 0.114 to 0.153 with a mean of 0.128. This figure corresponds closely with the mean figures obtained in this study in male control subjects and male patients with liver disease (0.125 and 0.121 respectively).

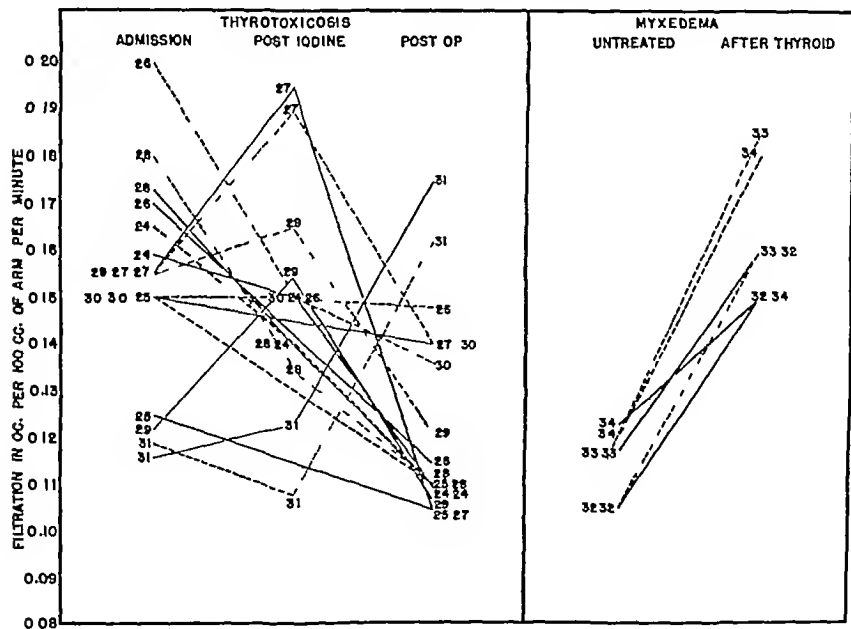


FIG. 2. RATES OF FILTRATION IN VARIOUS THERAPEUTIC STAGES OF THYROTOXICOSIS AND MYXEDEMA

Broken lines connect right arm readings, solid lines connect left arm readings. Note that in thyrotoxicosis there are wide fluctuations in the rate of filtration with a tendency to a reduction in rate after operative treatment. In myxedema there is a sharp increase in rate after thyroid therapy.

(15), by the technique of comparing the number of capillary tufts in an area of skin with the number observed in the same area after the nearby injection of histamine, concluded that in thyrotoxicosis the capillary bed was widely dilated. Shaw (16) measured the rate of blood flow in the arm in thyrotoxicosis by the use of the plethysmograph of Freeman, Shaw, and Snyder (17). Shaw made observations on patients with thyrotoxicosis before operation, after preparation with iodine, and one month or more following operation. He found in the majority of cases that the blood flow was most rapid during the toxic process, became less so following the administration of iodine and still less so after operation. However, as in the case of the rate of flow through the capillary walls, he found several notable exceptions in which the rate of blood flow increased following the administration of iodine. These observations roughly coincided with the changes which were noted in the rate of filtration through the capillary walls. It may be that the changes in filtration rate are directly dependent upon the degree of dilation of the capillary bed during the period of filtration. Wide dilation of the capillary bed produces an increase in the available surface of capillary endothelium and it may be that the increase in filtration rate observed in hyperthyroidism is dependent upon this factor rather than upon a specific change in the permeability of the capillary endothelium. This question cannot be answered by the pressure plethysmograph alone.

With regard to patients with myxedema at least two more uncontrolled factors are involved. After the myxedematous patients had been treated, their arm volumes became much smaller than previously, presumably because of diminution in the amount of edema. Landis and Gibbon showed that the rate of filtration depended in part upon tissue resistance. They showed that the presence of large amounts of tissue fluid slowed down the rate of filtration. Hence the slow rate of myxedema may be in part owing to increased tissue resistance from the viscous myxedema fluid. Another uncontrolled factor is the possibility of sudden changes in the level of serum albumin or globulin. The total protein level of patients with clinical myxedema was extremely high, and a high obstructing venous

pressure was applied to offset it. Unfortunately, the protein levels were not repeated after treatment, so that sudden falls in protein levels might have resulted in the use of an unjustifiably high obstructing venous pressure.

Whatever the underlying mechanism, there were very dramatic changes in the rate of filtration through the capillary walls of patients with hyperthyroidism and with myxedema at different basal metabolic levels. The close parallelism of the readings of the right and left arms confirms the significance of these changes.

CONCLUSIONS

1 In given individuals, repeated determinations of the rate of flow of fluid through the capillary walls could be checked with a reasonable degree of accuracy. The method is, therefore, readily applicable to studies where changes associated with other physiological variations in the same person are important.

2 At 35° C. the range of readings observed in normal controls was very wide and was not significantly exceeded in any of the pathological states studied.

3 The rates of filtration observed in patients with three different types of intrahepatic disease fell within the normal limits for their respective sexes. It is reasonable to conclude that abnormal accumulations of fluid in the tissue and serous cavities in liver disease is in no way dependent on alterations in capillary permeability.

4 Metabolic changes grossly affected the rate of flow of fluid through the capillary walls. Various mechanisms which may explain this phenomenon are discussed. The factor of oxygen consumption should be controlled in subsequent studies.

BIBLIOGRAPHY

1. Cutter I. S., and Marquart, G. H., Studies on capillary fragility. *Proc. Soc. Exper. Biol. and Med.*, 1930 28 113.
2. Cutter I. S. and Johnson, C. A., Studies on capillary fragility—a device for the study of capillary hemorrhage. *J. A. M. A.*, 1935 105 505.
3. Peterson W. F., The permeability of the skin capillaries in various clinical conditions. *Arch. Int. Med.*, 1927 39 19.
4. Landis, E. M., Microinjection studies of capillary permeability. I. Factors in the production of capillary stasis. *Am. J. Physiol.*, 1927 81 124.

THE LIPID DISTRIBUTION OF HUMAN PLATELETS IN HEALTH AND DISEASE¹

By BETTY N. ERICKSON, HAROLD H. WILLIAMS, IRA AVRIN, AND PEARL LEE

(From the Research Laboratory Children's Fund of Michigan and the Children's Hospital of Michigan, Detroit)

(Received for publication September 27, 1938)

It is well established that the platelets play an important rôle in blood coagulation (1). When exposed by bleeding they exhibit a characteristic physicochemical behavior (swelling, agglutination, excretion formation, and partial disruption (2)) resembling osmotic phenomena of phospholipids, e.g. myelin figure formation (3). In addition to serving physically in fibrin formation and clot retraction (2, 4), the platelets may liberate clot aiding substances (5). There is some debate as to the exact nature of the factors liberated by platelet disintegration. There can be no doubt as to a thermostable thromboplastic agent which has been identified with cephalin (5, 6, 7, 8), it is questionable whether there is, in addition, a coagulant factor, either of prothrombin like (1, 8) or different (5, 9) nature.

For a clearer definition of the *modus operandi* of platelets in blood coagulation, the present study has been focused upon their lipid composition in normal human blood and certain dyscrasias of blood coagulation in which platelet anomalies appear, namely, hemophilia and thrombopenic purpura. The data furthermore, may contribute information concerning the genealogical relationship of platelets to other formed elements of the blood as well as to cellular structure in general.

The chemical studies of the platelets have included total lipid, free cholesterol, cholesterol esters, neutral fat, total phospholipid, cephalin, and nitrogen.

METHODS

Postabsorptive blood samples (usually 50 cc.) were drawn from the arm vein into a syringe containing 10 cc. of chilled anticoagulant solution. The blood was delivered into ice-cold flasks and adjusted to twice the original volume by adding anticoagulant solution.

Upon the first three pooled samples collected, a 10 per cent sodium citrate solution was employed as the

platelet preservative. A sodium metaphosphate solution (2.0 per cent sodium metaphosphate, 1.0 per cent sodium chloride, and 0.2 per cent dextrose) proved more satisfactory (10) and was used for the later samples.

A suspension of intact platelets, virtually free of red and white blood cells was prepared by slow speed, differential centrifugation. The diluted blood was centrifuged in chilled, 15 cc., conical tubes for 10 minutes at 800 r.p.m. The diluted plasma containing some red and white cells was removed, placed in chilled tubes and centrifuged again at low speed (800 r.p.m.) for five minutes. By this time the platelet suspension could be decanted, leaving a small sediment of red and white cells. The centrifugations for 5 minute periods, and decantation into clean, chilled tubes was repeated 10 to 15 times until no visible red cell sediment was thrown down.

A microscopic examination was made at this point to assure the absence of contaminating red and white blood cells. The platelets at this stage, were intact, as indicated by the absence of clumping or gross changes in their appearance.

High speed centrifugation (2,000 r.p.m. for two hours) of the suspension enabled separation of the platelets as a white sediment. This precipitate was washed twice with chilled anticoagulant solution (diluted 1:2) by shaking and recentrifugation, and then dried *in vacuo* at low temperatures.

About 10 mgm. of dried platelet material were secured from each individual 50 cc. sample of blood. At least five individual platelet preparations were pooled for each nitrogen and lipid distribution analysis by the micro-gasometric methods of Van Slyke, *et al.* (11, 12).

CHEMICAL METHODS

The lipids were extracted from the pooled, dried platelet samples by heating under a reflux, on the water bath, with a 3:1 alcohol-ether mixture. The alcohol-ether extracts were evaporated *in vacuo* below 50°C. in an atmosphere of nitrogen (13). The residue was extracted with petroleum ether and total carbon and phosphorus determinations were made upon this extract by the micro-gasometric combustion procedures (12, 14). The phospholipids were precipitated from the petroleum ether extract according to the method of Bloor (15) and redissolved in moist ethyl ether. The phospholipids in this solution were determined upon aliquot portions

¹ Presented before the American Society of Biological Chemists 32d Annual Meeting, Baltimore, March 31, 1938. J. Biol. Chem., 1938, 123, xxxiv.

² A special grade of sodium metaphosphate (a soluble polymer) can be obtained from Howe and French Company, Boston.

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A microscopic examination was made at this point to assure the absence of contaminating red and white blood cells. The platelets, at this stage, were intact, as indicated by the absence of clumping or gross changes in their appearance.

High-speed centrifugation (2000 r.p.m. for two hours) of the suspension enabled separation of the platelets as a white sediment. This precipitate was washed twice with chilled anticoagulant solution (diluted 1:2) by shaking and recentrifugation, and then dried *in vacuo* at low temperatures.

About 10 mgm. of dried platelet material were secured from each individual 50 cc. sample of blood. At least five individual platelet preparations were pooled for each nitrogen and lipid distribution analysis by the micro-gasometric methods of Van Slyke, *et al.* (11, 12).

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TABLE II
Composition of formed elements in blood

	Platelets				Distribution of total lipid			
	Platelets	White cells*	Red cells	Red cell stroma	Platelets	White cells†	Red cells	Red cell stroma
	per cent of dry weight				per cent	per cent	per cent	per cent
Total lipid	15	22	1.3	11	74	60	58	67
Phospholipid	11		0.7	7				
Free cholesterol	2		0.3	2	16	16	23	20
Cholesterol esters	1		0.1	1	5	10	7	3
Neutral fat	1		0.2	1	4	14	12	10
Protein	64	69	90	56	68		58	65
Cephalin‡								

* From Haurowitz and Sladák (19)

† From Boyd (20)

‡ Per cent of total phospholipid

tenth as much lipid. However, the lipid content and distribution in red cells (calculated on a hemoglobin free dry weight basis), and in erythrocyte stroma, separately determined (19), is strikingly similar to that of platelets. The uniformity of the lipid patterns (Table II) may indicate a typical chemical structure and close biological interrelationship of the red and white blood cells and platelets.

It is necessary to examine the physicochemical state of the lipid and protein (19, 21) and their association within the cells, for an explanation of the individual behavior of the platelets, erythrocytes, and leukocytes, with respect to surface phenomena permeability and lability to osmotic lysis (22).

The platelets which are seriously diminished in congenital thrombopenic purpura, are often restored to a normal concentration by splenectomy (23). Platelets were secured from one patient, during a temporary stimulation to a record count of several million per cmm, soon after splenectomy. A single platelet sample taken when the count had passed the peak but was still at four million, provided sufficient material for a complete analysis (70 mgm of dried platelets from 50 cc.

of blood in contrast to a normal yield of about 10 mgm)

The lipid composition of the platelets in this sample of high platelet concentration (seven times normal) was not materially different from the composition determined in the samples from normal individuals (Table III). The apparent elevation of protein content may be caused by the use of sodium metaphosphate, rather than sodium citrate, as the anticoagulant.

TABLE III
Composition of human platelets in thrombopenic purpura after splenectomy and in hemophilia

	Thrombopenic purpura	Hemophilia			Normal children	Normal adults
		I	II	III		
Number of subjects	1	5*	4*	3†	9‡	25‡
Total lipid	12	12	15	15	13	16
Phospholipid	9	7	10	9	9	12
Free cholesterol	2	2	3	3	3	2
Cholesterol esters	0.1	1	1	0	0	1
Neutral fat	1	2	1	3	1	1
Cephalin§	66	69	63	66	66	68
Protein	74	72	76	69	56	66

* Clotting time 2 to 5 hours

† Clotting time 15 to 30 minutes.

‡ Sodium citrate used as anticoagulant for all samples from children and 15 of the adults. Sodium metaphosphate solution (10) used for all others.

§ Per cent of total phospholipid

The work of many observers has led to a suspicion that one of the anomalies in hemophilia lies in a functional ineffectiveness of the blood platelets (24, 25, 26). That the platelets do exhibit abnormal stability against disintegration has been demonstrated by dark field microscopic examinations (27). They apparently undergo the initial phenomena of osmotic alterations, including a modified excrescence formation, but they do not suffer rapid lysis (27). These observations have been confirmed in the hematological investigations being carried on in conjunction with the present studies (28). The disintegration of hemophilic platelets (determined by incubation for 6 hours at 38 C. in Olef's sodium metaphosphate solution) is negligible compared to the 40 per cent destruction of normal platelets. Differential platelet counts on the basis of size, initially and after incubation, demonstrate that the platelets are subject to measurable swelling (28).

Inasmuch as cholesterol and the various phospholipids are characterized by differences in their

* A 12 year-old girl who had been under the observation of one of the authors (P.L.) for four years preceding splenectomy. Following splenectomy the platelet count soon reached, and was maintained, at a normal level.

hydrophilic properties (29, 30), it is pertinent to search for clues to the anomalous behavior of hemophilic platelets within their own lipid constitution.

Nine individual platelet samples (combined as two samples for analysis) have been collected from the blood of five hemophiliacs⁵ with prolonged clotting times (2 to 5 hours). Three samples (pooled as one) were secured from three hemophiliacs⁵ with only slightly lengthened clotting times (15 to 30 minutes), but who had marked hemorrhagic symptoms and prolonged bleeding times. The hemophilic platelets possessed a normal quota of lipids (Table III) and showed no anomaly that could be detected by the present analytical methods.

On the basis of the lipid analyses it is of interest to calculate the amount of cephalin which *may* be contributed to the clotting mechanism by breakdown of the platelets. The platelets in 100 cc of blood could furnish from 5 to 10 mgm of cephalin (a concentration of one part in 10,000), if the entire amount in the platelets was liberated.

Extremely small amounts of "free" cephalin (of the order of one in several million) suffice for thrombin formation in the absence of excess antithrombin factors (31, 32). The significance of the observed amount of platelet cephalin depends upon the answers to the following queries: To what extent is blood clotting dependent upon platelet sources of phospholipids? How much of the cephalin is made "available" in the presence of the platelet proteins (9), plasma proteins (33), and electrolytes (2, 34)?

These analytical data are significant to the extent of proving that there is no lack of the clotting type of phospholipids in the blood platelets of normal and hemophilic humans.

SUMMARY

Platelet samples from the blood of healthy men, women, and children, hemophiliacs, and a patient with thrombopenic purpura (following splenectomy) were collected and analyzed by micro-gas-

metric methods for total lipid, total phospholipid, cephalin, free cholesterol and cholesterol esters, neutral fat, and nitrogen.

Lipid values (per cent of dry weight) on four pooled platelet samples (composite of five or more) from 25 adults, were uniform and averaged as follows: Total lipid, 16; phospholipid, 12; free cholesterol, 2; cholesterol esters, 1; neutral fat, 1 per cent. Cephalin composed 68 per cent of the total phospholipid.

The platelets from the blood of normal children (one sample made up of 9 individual collections) demonstrated a slightly lower value for phospholipid.

In hemophilia, the platelets appeared to possess normal amounts of the lipid constituents (12 samples pooled as 3 for analysis, and separated on the basis of clotting time).

The platelets secured from a patient with thrombopenic purpura, during the stimulated phase following splenectomy, exhibited a normal proportion of lipid constituents.

Similarities in the lipid composition of human platelets, leukocytes, erythrocytes, and stroma were pointed out and discussed.

BIBLIOGRAPHY

- 1 Howell, W. H., Theories of blood coagulation. *Physiol. Rev.*, 1935, 15, 435.
- 2 Ferguson, J. H., Observations on the alterations of blood platelets as a factor in coagulation of the blood. *Am. J. Physiol.*, 1934, 108, 670.
- 3 Leathes, J. B., Role of fats in vital phenomena. *Lancet*, 1925, 1, 803, 853, 957, 1019.
- 4 Tocantins, L. M., Platelets and the structure and physical properties of blood clots. *Am. J. Physiol.*, 1936, 114, 709.
- 5 Mills, C. A., The role of platelets in blood clotting. *Chinese J. Physiol.*, 1927, 1, 235.
- 6 Eagle, H., Studies on blood coagulation, role of prothrombin and of platelets in the formation of thrombin. *J. Gen. Physiol.*, 1935, 18, 531.
- 7 Chargaff, E., Bancroft, F. W., and Stanley-Brown, M., Studies on the chemistry of blood coagulation. The chemical constituents of blood platelets and their role in blood clotting, with remarks on the activation of clotting by lipids. *J. Biol. Chem.*, 1936, 116, 237.
- 8 Ferguson, J. H., An experimental analysis of coagulant activation. *Am. J. Physiol.*, 1936, 117, 587.
- 9 Mills, C. A., Do blood platelets, plasma, and tissues yield thrombin or tissue fibrinogen? *Am. J. Physiol.*, 1930, 95, 1.

⁵ The diagnosis of hemophilia in these individuals (ages 5 to 13 years) was established beyond question by clinical and laboratory observations over a period of several years (P.L.).

- 10 Olef I., The rate of disintegration of platelets. *J Lab and Clin. Med.*, 1936 22, 128.
- 11 Peters, J P and Van Slyke, D D., Quantitative Clinical Chemistry Vol. II Methods. Williams & Wilkins Company Baltimore, 1932, p 353
- 12 Kirk, E., Page, I. H., and Van Slyke, D.D., Gasometric microdetermination of lipids in plasma, blood cells and tissues *J Biol. Chem.*, 1934 106, 203
- 13 Williams H H Erickson B N., Avrin, I., Bernstein, S S and Macy I G Determination of cephalin in phospholipids by the estimation of choline. *J Biol. Chem.* 1938, 123 111
- 14 Kirk, E., Gasometric microdetermination of phosphoric acid. *J Biol. Chem.*, 1934 106 191
- 15 Bloor W R., The oxidative determination of phospholipid (lecithin and cephalin) in blood and tissues. *J Biol. Chem.*, 1929 82, 273
- 16 Erickson, B N., Williams, H. H., Hummel F C. and Macy I. G., The lipid and mineral distribution in the serum and erythrocytes of normal children. *J Biol. Chem.*, 1937 118 15
- 17 Haurowitz, F and Sladé, J., Über die Chemische Zusammensetzung der Blutplättchen. *Ztschr f physiol. Chem.*, 1928 173 233
- 18 Bernstein, S S., Jones R. L., Erickson, B N., Williams H. H., Avrin, I., and Macy I G., A method for the preparation of posthemolytic residue or stroma of erythrocytes. *J Biol. Chem.*, 1938, 122, 507
- 19 Erickson, B N., Williams, H H., Bernstein, S S Avrin I., Jones R. L., and Macy I G., The lipid distribution of posthemolytic residue or stroma of erythrocytes. *J Biol. Chem.* 1938 122, 515
- 20 Boyd, E. M., The lipid content of the white blood cells in normal young women. *J Biol. Chem.*, 1933 101 623
- 21 Beach, E. F., Erickson B N., Bernstein S S., and Williams H H., Basic amino acid content of posthemolytic residue or stroma of erythrocytes. *J Biol. Chem. (Proc.)* 1938 123, vi.
- 22 Silberberg N., The causes and mechanism of thrombosis *Physiol. Rev.*, 1938 18 197
- 23 Brown, D N., and Elliott, R. H E., The results of splenectomy in thrombocytopenic purpura. *J A. M. A.*, 1936, 107 1781
- 24 Minot G. R. and Lee, R. I. The blood platelets in hemophilia. *Arch. Int. Med.* 1916 18, 474
- 25 Howell W H. and Cekada, E. B., The cause of the delayed clotting in hemophilic blood. *Am. J Physiol.*, 1926 78 500
- 26 Christie, R. V., Davies, H W., and Stewart, C. P., Studies in blood coagulation in haemophilia. I Blood coagulation in hemorrhagic diseases. Observations on haemic functions in haemophilia. *Quart. J Med.*, 1926-27 20, 481
- 27 Harrow B and Sherwin C. P., Textbook of Biochemistry Chap. XV Blood—Ferguson J H. W B Saunders Co., Philadelphia, 1935
- 28 Lee, Pearl and Erickson Betty N., Platelet studies in normal men and women (menstruating and non menstruating) and subjects with bleeding disorders Counts, disintegration rates and intradermal platelet injections. *Proc. Soc. Exper Biol. and Med.* (In press.)
- 29 Maclean, H. and Maclean, I S., Lecithin and Allied Substances—the Lipins. Longmans Green and Co. London, 1927
- 30 Bull, H B., The Biochemistry of the Lipids. Burgess Publishing Co., Minneapolis 1935
- 31 Spagnol G. Cephalins and blood coagulability *Rev. sud. am. de endocrinol.*, 1934 17, 619 *Chem. Abst.*, 1935 29 226.
- 32 Ferguson, J H Quantitative relationships of calcium and cephalin in experimental thrombin formation. *Am. J Physiol* 1938 123, 341
- 33 Page, I H., Chemistry of the Brain. Charles C. Thomas Baltimore, 1937 p. 278
- 34 Ferguson J H., The blood calcium and the calcium factor in blood coagulation. *Physiol Rev* 1936 16, 640

THE NATURE OF THE HUMAN FACTOR IN INFANTILE PARALYSIS I PECULARITIES OF GROWTH AND DEVELOPMENT¹

By GEORGE DRAPER AND C. WESLEY DUPERTUIS

(From the Department of Medicine College of Physicians and Surgeons Columbia University and the Presbyterian Hospital New York City)

(Received for publication October 7 1938)

Since the great epidemic of infantile paralysis in 1916 research in every phase of the subject has been actively maintained. Explorations into the nature of the virus, portal of entry, epidemiology, prevention, and treatment have been vigorously pursued. In the Constitution Clinic of the Presbyterian Hospital our interest has been concerned especially with the evaluation of the personal identity or constitutional characters of the paralyzed child or adult. The present research was originally undertaken in 1916 for the following reasons. First the general principle that Human Disease represents a conflict between a living individual and some specifically adverse element of its environment. Second, that in the most heavily infected areas many children of susceptible age remained well, third that in the presence of an identical sample of virus there was great variation in the severity of the clinical course, fourth that there was a notable difference between the morphology of stricken and well children.

Our observations upon these constitutional differences have been published from time to time since their first mention in 1917 (1, 2). As in our previous studies the morphology of stricken persons has been our initial avenue of approach to the questions "Are the subjects of any given disease constitutionally different and recognizable from those individuals who escape that particular malady?" A dissenting view of this thesis in the case of infantile paralysis is taken by Levine, Neal, and Park (3) and also by Thelander and Pryor (4). It is interesting to note, however that earlier clinicians mentioned that poliomyelitis victims were notable for their large size, robustness, and sound teeth. Thus Underwood (5) in 1799 referred to "fine children" and in 1823 Shaw (6) wrote "strong and healthy children are more frequently affected than those of a

weakly constitution." There are many other similar statements by other authors, including Aycock (7) who also has observed differences between the constitutional types of stricken and well subjects. From these writings it would appear that the notion of a "host factor" in acute poliomyelitis was stirring as early as 1799, perhaps even before the infectious idea was born. Furthermore, since morphology is clearly, at least in part, an inherited character, the question has often been asked whether other qualities such, for example, as immunity might likewise be a genetic one. The recent work of Webster (8, 9) indicates that certain breeding techniques markedly influence the resistant or susceptible factor in some strains of mice to both bacterial and virus infections. "Heredity" he states "has proved clearly to be an element of fundamental importance in determining the fate of individuals following primary exposure to a natural infection." And again, in a subsequent paper "The thesis of variability of host resistance and its regulation by inborn and environmental factors has both particular and general bearing upon experimentation in infectious disease. Again the role of innate resistance factors is being investigated in experimental epidemiology, for example, in the matter of determining the status of survivors of an epidemic. Are they inherently resistant at the outset and spared from the ravages of the epidemic agent, or are they differentiated only by the chance exposure to subinfectious doses which have immunized them, or do both processes participate?"

Furthermore so far as infantile paralysis is concerned, we have evidence in our clinic (not reported), and numerous other observers notably Aycock (10) have published genetic histories which indicate beyond doubt that the disease may well "run in families."

The purpose of this communication is to support further our contention that

¹ The work reported in this communication was carried out under a grant from the Rockefeller Foundation.

tible to the adverse effects of poliomyelitis virus are highly specialized types of the human race, that they possess a recognizable physical constitution which is determined by inherited faults or adverse intrauterine environment, or both. These result in growth and development irregularities, retardations, and endocrine imbalance of a definite character which appear to correlate with susceptibility to the virus.

MATERIAL

Measurements and observations upon 148 paralyzed boys, 125 paralyzed girls, and a control group of 229 "well boys" all between the ages of 5 and 20 compose the material of this investigation. There is no control series of well girls, but it turned out that the paralyzed females were similar to the paralyzed males (excepting primary sex characters) in all measurable and non-measurable (observed) characters. To facilitate the statistical reduction of the data, the series were divided into age groups of 2-year intervals. Ages 15 to 20, because of paucity of numbers were combined in one unit. In the male paralyzed series, the number of cases in each group varied from 20 to 33, in the control series from 20 to 57, in the female paralyzed series 16 to 26. Without regard to the time they were attacked the subjects have been grouped according to their age at the time they were measured.

The racial factor was carefully studied. But after adding to our New York material a series of 31 (both sexes) paralytics in Sweden and 27 cases in Finland,² and a large control group of well boys and girls from Swedish summer camps, we conclude that among white people, at least, race apparently plays a negligible role in susceptibility to infantile paralysis.

We have continued to apply the technique of measurement and observation which we have pre-

viously employed, and have subjected the data to statistical analysis. It has been surprising how consistently the figures have supported our earlier contentions that the poliomyelitic susceptible was a specific and recognizable type. But, in addition, the calculations have brought out some new concepts of the possible nature of this poliomyelitic susceptible human host type. One of these involves the problem of growth and development, another has to do with age (time factor) in relation to the distribution and severity of paralysis.

Because the statistical material is so extensive, we have thought it best to present the work in two sections, the first discussing the findings in respect to growth and development, the second those related to age and paralysis. But, it should be understood that because of the complex interrelationships of the two sets of phenomena, a sharp division is impossible.

MEASUREMENTS

The most notable finding is that paralyzed boys in the age group 5 to 8 and from 13 on possess larger head and face size than do the controls. Between the ages of 9 and 13 the sick and well groups present no differences. But again in the 15 to 20 age group the cripples display significantly greater dimensions in 14 out of 27 measurements. The interpupillary distance and the breadth between the inner canthi is likewise greater among the sick individuals of the earlier and later age groups. Furthermore, their eye slits are longer than those in the well series.³

When the entire series is divided into the 2-year age groups clear differences in comparative size appear between those which precede and follow puberty. This finding we have considered as a difference in growth rhythm between the two groups (Figure 1). In the later age groups body measurements indicate somewhat greater size among afflicted persons. On the other hand, for a character such as cephalic index we have never found any correlation with types susceptible to infantile paralysis.

² It gives us great pleasure to express our thanks to Prof. Carl A. Kling, Director of State Immunity Institute, Sweden, Dr. Severin, Director of Boys' Camp, Reisingbo, Sweden, and to Dr. Gunnar Svedenius, Medical Director of Girls' Camp, Barnens Ö, Sweden, for their interest and cooperation in helping us to get access to their very rich material. Without their assistance, we would not have been able to include the material from Scandinavia and Finland. To Dr. Wertsanen of Lomaa, Finland, we are likewise greatly indebted for putting his cases at our disposal.

³ The detailed data which are available in this laboratory and are not included in this paper because of their magnitude give the differences of the means of all the measurements and indices with the value in terms of the probable error for each age group.

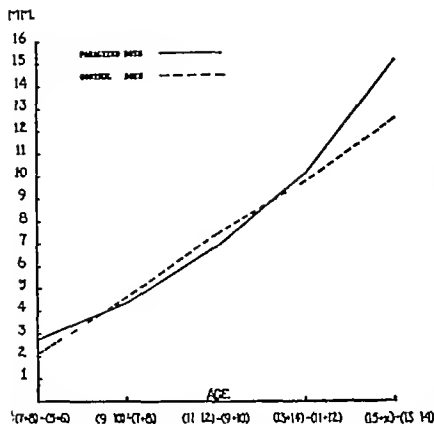


FIG. 1 CURVE SHOWING ACCUMULATED BIENNIAL INCREMENTS OF GROWTH

The increments express the difference, each 2 years between the means in millimeters of the sum of all linear measurements.

To recapitulate then, it appears that afflicted children tend to be larger in size during the years before and after puberty. But during the time period commonly allocated to the establishment of sexual maturity there is no demonstrable difference in growth achievement between the sick and well groups. Now when we consider the non-measurable characters a somewhat different picture confronts us. These phenomena which now present themselves for discussion seem to be related almost exclusively to the function of development. They comprise details of design and tissue quality some of which tend in general to be modified or to disappear with advancing years.

OBSERVATIONS

We have chosen six characters from among a large number of the observable ones which show significant differences between sick and well groups. These six were chosen because they have been so consistently emphatic. Indeed we have come to rely on them as dependable criteria of the poliomyelitic susceptible constitution. Table I shows the excesses in the frequency (percentage) of these characters in the sick over the well male groups. These differences are also expressed in

TABLE I

Differences of percentages in the six characters between the sick and well groups with values in terms of probable error Sick boys/Well boys

	Difference per cent	xpe
Black spots—Present	+23.05	+ 8.60
Eye lashes—Long curved	+38.33	+11.87
Central incisor teeth—Large	+30.57	+ 8.81
Hyperextension of joints—Pronounced	+26.44	+ 7.73
Central incisor spacing—Present	+17.33	+ 4.96
Internal eye folds—Present	+24.39	+ 7.28

terms of their probable errors (If the xpe is above 3 the difference is significant.)

Pigment spots The physiology of pigment in health and disease is not yet fully understood. Consequently it is not possible now to offer a suggestion as to its significance in patients who are susceptible to infantile paralysis. Our findings indicate two things. First irregularity of pigmentation is definitely more extensive and frequent in our paralyzed series than in the controls. Second this difference seems to be entirely independent of eye color, hair color, and race.

TABLE II

Black spots—Male series

	Absent		Present		Total	
	Num ber	Per cent	Num ber	Per cent	Num ber	Per cent
Sick total	15	10.14	133	89.86	148	100.00
Control total	76	33.19	153	66.81	229	100.00

Large central incisors and central incisor spacing Our figures disclose a greater amount of large and irregular dentition in the sick group. Believing as we do that the manner of dental eruption expresses an important index of the total organism's growth and development, tooth form and placement are for us matters of constitutional significance.

TABLE III

Size of central incisor teeth—Male series (Ages 7 and above only)

	Small and average		Large		Total	
	Num ber	Per cent	Num ber	Per cent	Num ber	Per cent
Sick total	21	16.80	104	83.20	125	100.00
Control total	99	47.37	110	52.63	209	100.00

TABLE IV
Central incisor spacing—Male series

	Absent		Present		Total	
	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent
Sick total	70	47.30	78	52.70	148	100.00
Control total	148	64.63	81	35.37	229	100.00

Eyelashes In the control group eyelashes tend to grow shorter with advancing years. In the paralyzed group, on the other hand, there is no shortening as they grow older. We have as yet no explanation for this phenomenon. But it is again one which displays a different conduct in relation to increasing maturity between the sick and well groups.

TABLE V
Eyelashes—Male series

	Short or medium		Long curved		Total	
	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent
Sick total	26	17.57	122	82.43	148	100.00
Control total	128	55.90	101	44.10	229	100.00

Hyperextensibility of joints Hyperextensibility of hand and fingers is a universal character of human infants. It disappears at a varying rate with advancing years until a sharp drop occurs at about 13 years of age. Among infantile paralysis patients, however, there is a much higher percentage of hyperextensibility in all age groups. But the curve of descent practically parallels that of the control group.

TABLE VI
Hyperextensibility of joints—Male series

	Absent to medium		Pronounced		Total	
	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent
Sick total	52	35.13	96	64.87	148	100.00
Control total	141	61.57	88	38.43	229	100.00

Internal eyefolds (Epicanthic, "Mongoloid") The three well known characters of the Mongol eye are 1, Inner canthus lower than outer giving

a slant to the palpebral fissure, 2, a smooth downward and inward curve of the upper lid margin, 3, the epicanthal fold of skin which sweeps down almost vertically over the inner canthus. Various elements of this trio or all of them are not infrequently seen in members of the white race. This eye construction is usually associated with a flat nose bridge and wide inter-inner canthus space.

TABLE VII
Internal eyefolds—Male series

	Absent		Present		Total	
	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent
Sick total	77	52.03	71	47.97	148	100.00
Control total	175	76.42	54	23.58	229	100.00

Our findings show this character in one or more of its constituent forms to be present far more often among the paralyzed than the well. Furthermore, there were three Mongoloid idiots in Willard Parker Hospital suffering from acute poliomyelitis during the 1931 epidemic. The population incidence of such arrested types is 1 in 1000 of child population. The cause of Mongolism is not known. But this peculiar condition is now thought to be caused by some adverse intrauterine influence which results in a special form of retardation in growth and development. Disturbance of anterior pituitary lobe, adrenal cortex, and gonads have been suggested. It is also worth noting that the epicanthal fold, flat nose bridge, and wide inter-inner canthus space, and wide set eyes is almost typical of the white fetus.

The marked difference of facial design between fetus, infant, and adult is well known and depends largely on elevation of the nose bridge. Our figures show, for example, that the inter-inner canthus breadth is significantly greater for the patients than the controls, for the age groups 5 to 6 and 7 to 8 years. This difference between sick and well group is not so marked at puberty. But it persists as one of the insignia of retardation in many older paralyzed children to later ages than the controls.

While the two methods—mensuration and observation—are obviously only applicable in their appropriate forms, the value of each method can

naturally intimately interwoven. Table VIII derived from stricken girls over 7 years of age is offered as an example of the interrelation. Should we take for example the figure which represents the ratio 'interpupillary space over facial diameter', this whole group can be divided into two—one of which displays indices below 47 the other 48 and above. If now the members of these two groups be classified according to the number of the six characters each possesses the structure of Table VIII becomes clear. The figures show apparently that when the index is low (i.e. below 47) 80 per cent of such individuals possess only one of the six characters. When the index is high (above 48) on the other hand the individuals tend to present an increasing number of the six characters. This would again indicate that with increase in size (growth) there is found an increase in criteria of inadequate development.

TABLE VIII
Paralyzed girls ages 7 and over
Interpupillary space/Facial diameter

Number of characters	Low index,* up to 47	High index,* 48 and over
	per cent	per cent
I (One character)	80.00	20.00
II (Two characters)	57.14	42.86
III (Three characters)	77.78	22.22
IV (Four characters)	55.56	44.44
V (Five characters)	48.57	51.43
VI (Six characters)	36.36	63.64

* Low index = narrow eye set relative to face breadth
High index = wide eye set relative to face breadth

In the first two age groups we have seen the analogue of the fetal face, suggesting a retardation in the metamorphosis of this important facial area (Figure 2). The term retardation here does not refer to size, for often these retarded faces are large. It refers to the degree of maturity. The subsequent pushing forward of the nose bridge with advancing months and years rapidly changes the fetal and infant aspects in the direction of adult nasalization. In the age groups 9 to 13 these special eye nose zone characters show little or no difference between sick and well. But from then on the fetal and infantile resemblances reappear in subtly varying degree among the paralyzed group. It is as if the sick group were capable to some extent of pushing the nose bridge forward from the original fetal eye and nose design. But it only goes so far. On the other



FIG. 2 BOY OF 7 SHOWING FACIAL CHARACTERS OF POLYMYELIC SUSCEPTIBLE TYPE

Note excessive inter inner canthus space and Mongoloid fold also black spot on left chest. This boy also has widely separated upper central incisors flat nose bridge, and wide set eyes.

hand the well group at this point maintain the continuing nasal growth process appropriate to the 9 to 12 age level. Then with the puberty thrust they forge ahead. The sick group are left behind and remain fixed at some point of retardation. So far as eye nose zone is concerned such retardation is more notable by contrast as the rest of the face grows more mature.

Another phenomenon which presents a somewhat similar picture of discrepancy between advance in general body growth and functional retardation is found in the external genitalia of male paralytics. Not only are small organs almost the rule among the sick group but partial or complete cryptorchidism is significantly more common in the stricken than in the resistant persons. Coincident with the retardation in gonad development it was observed that the body build in the paralyzed boys tended more towards the curved feminine (perhaps better the human species type which underlies that of both sexes) as compared with the more angular masculine.

body build of the well boys. The inverse ratio of large body size and small gonads again points to a lack of parallelism between the two important phases of the maturing process.

It should be evident from the foregoing paragraphs that we are confronted with the two complex and intimately associated phenomena of growth and development. They seem to be as definitely related to the unsuccessful maturing of a specifically ill person as they may be to the final completion of a healthy specimen. We have considered that the two words "Growth" and "Development" have different connotations but that the processes they describe are importantly related, indeed perhaps merged. Growth for us means augmentation in size. Development on the other hand connotes the continuous modification of an individual's total life processes which transpire between the egg stage and adult form. It deals not with size but with events of functional rearrangement and especially with the time at which these events occur, and their relation to one other in respect to the time of their occurrence.

Furthermore, since both growth and development express energy in motion their relationship with time determines rate. The importance of the rate of growth and development of the parts of an organism is well known to be a matter of profound significance to the successful completion of the adult phenotype. Stockard's (11) experiments on the production of various degrees of twinning in trout by retarding the metabolism rate of germinating eggs is an excellent illustration of the importance of pace for maturing embryos. Newman (12) has published somewhat similar studies.

Our findings would seem to show that the susceptibility to the virus of infantile paralysis is part of a definite type of faulty constitution. As in all other biological phenomena there is a wide range in the degree, but we believe that there is adequate support for the notion that poliomyelitic susceptibles are different from the resistant persons. These differences are doubtless relative and as subtly relative as are the merging tones of the chromatic scale in music. From this it might be inferred that the number and degree of the characters—the legibility of the stamp as it were—may correlate positively with the severity of the disease—whether abortive or paralyzed, including the latter's distribution and extent.

It is interesting that anthropometry demonstrated less evidence of difference between sick and well than did the observations of non-measurable characters. Possibly, the explanation for this lies in the fact that growth (in the sense of size augmentation) is not perhaps so closely involved with susceptibility. Resistance to infection on the other hand is more intimately related to the changing functions of metamorphosing cells. And so it would seem as though there were a splitting of the two forces which carry the organism to completion. Among poliomyelitic susceptibles there is a tendency to overgrowth and underdevelopment. The latter persists while growth continues and emphasizes the developmental retardation. Among non-susceptibles, growth and development proceed more in step with each other and with age (time appropriateness). These growth irregularities and developmental retardations, as well as the sex difference in the attack rate suggest adverse genetic, or intrauterine forces, and later endocrine unbalance as being responsible for the type susceptible to infantile paralysis.

CONCLUSIONS

1. A method composed of mensuration, observation, and statistical analysis has been used for studying the external morphology of subjects with infantile paralysis.

2. Persons susceptible to the virus of acute anterior poliomyelitis possess a special constitutional type of morphology which differs significantly from that of non-susceptibles.

3. Variations occur in the degree of the morphological differences extending from nearly imperceptible ones to those of high statistical significance. This scale may parallel the severity of the individual illness, and likewise possess epidemiological significance.

4. Among susceptibles there is a lack of coordination between growth and development. This is expressed in a tendency to overgrowth and retarded development.

5. The peculiar presence of the Mongoloid eye and the fetal and infant-like retardation of the eye-nose zone suggest adverse genetic or intrauterine forces.

6. The person susceptible to infantile paralysis is a different and in some way incomplete phenotype, the incompleteness depending upon certain

faulty genetic characters or adverse intrauterine events which alter the time relationships in the processes of growth and development.

ADDENDA

Following is a list of the anthropological measurements and indices derived from them

Cephalic length	Biliac diameter
Cephalic breadth	Thoracic lateral diameter
Facial diameter	Thoracic A.P. diameter
Bigonial diameter	Webb
Facial height	Cephalic index
Nasion prosthion	Facial index
Nasal height	Upper face index
Nasal breadth	Nasal index
Infradentale-menton	Ear index
Ear length	Palpebral index
Ear breadth	Nail index
Interpupillary space	Hand index
Palpebral length	Thoracic index
Palpebral breadth	Biliac diameter/ Biacromial diameter
Inter inner canthus	Interpupillary space/ Nasion prosthion
Nail length	Interpupillary space/ Facial diameter
Nail breadth	Bigonial diameter/ Facial diameter
Finger length	Inter inner canthus/ Facial diameter
Hand length	
Palm length	
Palm breadth	
Gonial angle	
Biacromial diameter	

BIBLIOGRAPHY

1. Draper, G., *Acute Anterior Poliomyelitis*. P. Blakiston's Son and Co., Philadelphia, 1917
2. Draper, G., The nature of the human factor in infantile paralysis. *Am. J. M. Sc.*, 1932, 184, 111
3. Levine, M. L., Neal, J. B., and Park, W. H. Relation of physical characteristics to susceptibility to anterior poliomyelitis. *J. A. M. A.*, 1933, 100, 160
4. Thelander, H. E., and Pryor, H. B., Anthropometric and anthroposcopic studies of poliomyelitis. *Arch. Pediat.*, 1933, 50, 749
5. Underwood, Michael, *Treatise on Diseases of Children*. London, 1799
6. Shaw, J., *On the Nature and Treatment of the Distortions to which the Spine and the Bones of the Chest are Subject*. Longman Hurst, London, 1823
7. Aycock, W. L. Nature of autarceologic susceptibility to poliomyelitis. *Am. J. Pub. Health*, 1937, 27, 575
8. Webster, L. T., Inherited and acquired factors in resistance to infection. *J. Exper. Med.*, 1933, 57, 793.
9. Webster, L. T., Inheritance of resistance of mice to enteric bacteria and neurotropic virus infections. *J. Exper. Med.*, 1937, 60, 261
10. Aycock, W. L., Autarceology of poliomyelitis. *West Virginia M. J.*, 1934, 30, 481
11. Stockard, C. R., Developmental rate and structural expression. *Am. J. Anat.*, 1931, 28, 115
12. Newman, H. H., On the production of monsters by hybridisation. *Biol. Bull.*, 1917, 32, 306.

THE NATURE OF THE HUMAN FACTOR IN INFANTILE PARALYSIS II RELATION OF AGE TO MATURING ACHIEVEMENT AND THE DISEASE PICTURE¹

BY GEORGE DRAPER AND C. WESLEY DUPERTUIS

(From the Department of Medicine College of Physicians and Surgeons Columbia University and the Presbyterian Hospital New York City)

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In the first paper of this series the susceptible human organism's state of growth and development was under discussion, and the retarded or incomplete phenotype which seemed to correlate highly with susceptibility was compared with well children (1)

The present paper attempts to explore the genetic and constitutional basis of the difference between sick and well, the relation of various age levels to susceptibility, and to the location of paralysis

It would seem that the ages of 6 to 7 (second dentition) and that of 11 to 15 (puberty) are more or less critical ones in the organism's march to completeness. The controls pass through these time points without much change in their growth rates. The poliomyelitics on the other hand seem to be in some way stimulated to a sudden size increase following the achievement of each of these two age levels. But this stimulus does not serve to advance their already retarded development (see (1), p 13). The controls apparently either receive no such stimulus or do not react to it. Their growth and development processes move on in parallel, undisturbed and as a more average continuum.

Age of onset We are ever impressed by the relation between growth, development, age, and the moment of disease onset. There is here, however a danger of possible confusion in the fields of cause and effect. It might be said for example that the disease itself provokes some change in the growth and maturing processes which result in the organism's failure to achieve completeness. In upholding our belief, however, that these 4 phenomena just mentioned are genetic, truly constitutional ones, and not conditioned by the disease, we can refer first to the fact that "style" of organ development is definitely

hereditary. Thus the form and position of teeth are due to that same cause whether spaced or close-set. Moreover, there is a fairly high percentage (42.67 per cent) of close-set teeth among those children who became ill before the eruption of the second dentition. Tables I and II form the basis for these views.

These tables indicate that there are no significant differences in frequency of black spots, large central incisors, long curved eyelashes or internal eyefolds between those children who became paralyzed before 7 years of age and those who were afflicted at a later age. On the other hand there are significant differences between the two age groups in only two of the six characters namely, central incisor spacing and pronounced hyperextensibility of the joints. The higher percentages for these two traits are found among those children who became ill during the first 6 years of life. But it was pointed out in the first paper that these characters tend to disappear with increasing years in both sick and well the former more slowly and often less completely than the latter. As a result of these observations, it would seem that the specific characters just discussed are marks of an inherited special constitutional type and not products of the disease. Furthermore a fair percentage of all the characters are found also among the control group. We conclude from the above that paralyzed children no matter at what age they were measured and observed, have possessed those characters from birth.

In our discussion of the six characters which were found with much greater frequency in the sick group, we took into consideration only the percentage distribution of these characters as compared to the control series. It may well be of equal importance to examine the quantitative distribution of these characters among the sick and the well. In Table III and the subsequent tables the Roman numerals refer to the

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TABLE I

Percentage distribution of characters according to age at onset of disease Paralyzed boys Age 7 and over (when measured)

Age of onset	Number		Per cent			
Before 7 years	75		60 00			
7 years and above	50		40 00			
Black spots						
Age at onset	Absent		Present		Total	
	Num ber	Per cent	Num ber	Per cent	Num ber	Per cent
Before 7 years	5	6 67	70	93 33	75	100 00
7 years and above	1	2 00	49	98 00	50	100 00
Eyelashes						
Age at onset	Short and medium		Long curved		Total	
	Num ber	Per cent	Num ber	Per cent	Num ber	Per cent
Before 7 years	10	13 33	65	86 67	75	100 00
7 years and above	14	28 00	36	72 00	50	100 00
Hyperextensibility of joints						
Age at onset	Absent to medium		Pronounced		Total	
	Num ber	Per cent	Num ber	Per cent	Num ber	Per cent
Before 7 years	24	32 00	51	68 00	75	100 00
7 years and above	27	54 00	23	46 00	50	100 00
Central incisor spacing						
Age at onset	Absent		Present		Total	
	Num ber	Per cent	Num ber	Per cent	Num ber	Per cent
Before 7 years	32	42 67	43	57 33	75	100 00
7 years and above	35	70 00	15	30 00	50	100 00
Internal eyefolds						
Age nt onset	Absent		Present		Total	
	Num ber	Per cent	Num ber	Per cent	Num ber	Per cent
Before 7 years	41	55 41	33	44 59	74	100 00
7 years and above	29	58 00	21	42 00	50	100 00
Large central incisors						
Age at onset	Absent		Present		Total	
	Num ber	Per cent	Num ber	Per cent	Num ber	Per cent
Before 7 years	12	16 00	63	84 00	75	100 00
7 years and above	12	24 00	38	76 00	50	100 00

TABLE II

Differences of percentages of observations on paralyzed boys who became sick before 7 years of age and those who became afflicted at 7 years or after with value in terms of probable error

Age of onset before 7 years/Age of onset 7 years or above

	Difference	xpe
1 Black spots—Present	- 4 67	-1 98
2 Eyelashes—Long, curved	+14 67	+2 92
3 Hyperextensibility of joints— Pronounced	+22 00	+3 68
4 Central incisor spacing—Present	+27 33	+4 70
5 Internal eyefolds—Present	+ 2 59	+ 42
6 Large central incisors—Present	+ 8 00	+1 61

TABLE III

Table showing percentage distribution of six characters among sick boys and girls and control boy series

	Num- ber	Per cent
Paralyzed boys—Ages 7 and over		
VI Six characters present	10	8 00
V Five characters present	40	32 00
IV Four characters present	39	31 20
III Three characters present	27	21 60
II Two characters present	9	7 20
I One character present	0	0
0 None present	0	0
	125	100 00
Summary		
3 or more characters present	116	92 80
4 or more characters present	89	71 20
2 or less characters present	9	7 20
Paralyzed girls—Ages 7 and over		
VI Six characters present	17	15 89
V Five characters present	29	27 10
IV Four characters present	30	28 04
III Three characters present	18	16 82
II Two characters present	7	6 54
I One character present	5	4 67
0 None present	1	93
	107	99 99
Summary		
3 or more characters present	94	87 85
4 or more characters present	76	71 03
2 or less characters present	13	12 15
Control series, boys—Ages 7 and over		
VI Six characters present	3	1 44
V Five characters present	16	7 66
IV Four characters present	32	15 31
III Three characters present	55	26 32
II Two characters present	65	31 10
I One character present	30	14 35
0 None present	8	3 82
	209	100 00
Summary		
3 or more characters present	106	50 72
4 or more characters present	51	24 40
2 or less characters present	103	49 28

six characters possessed by the members of each group. Thus, for example, the numeral VI means that all six of the chosen characters are present. On the other hand II represents those individuals who possess only two of the six characters.

A glance at the summaries shows for example that among the sick boys, 72.0 per cent possess only two characters or less, whereas among the controls 49.28 per cent have two or less. Furthermore among the stricken, 92.8 per cent have three or more of the six characters and the well who possess three or more form but 50.72 per cent. The figures for girls parallel those of the boys very closely.

There is also a correlation between the number of characters a child shows and the age at which he is measured. That is, those individuals in the male paralyzed group, for example, who have the least number of the six characters are among the oldest. Those who have the largest number of characters tend to be the youngest. This is true also for the control group. The demonstration of this fact is represented by the coefficient of mean square contingency for age (at the time of measurement) and number of characters. The figures are 0.56 for the sick boys and 0.51 for the control group of well boys. In other words, if age and number of characters be correlated it appears that the sick individuals average more of the six characters than the well, but their loss in relation to increasing ages follows a parallel course. If we take into account, however, the age at which these sick children became paralyzed we have a somewhat different picture. We have found, for example, that of the group of 9 sick children (Table IV) who exhibited only two of the six characters none were below 11 years of age when measured. Seven of these 9 children, however, got the disease at 7 years of age or later and only 2 before 7 years. Of the 10 boys who exhibited all six characters, 8 or 80 per cent got the disease before 7 years of age and only 2 or 20 per cent at 7 years of age or after. The figures for the girls are even more striking (Table V).

From the foregoing, then, it appears that even though there is a correlation between age (when measured) and the number of characters possessed by these paralyzed children we find also a

TABLE IV
Paralyzed boys—Ages 7 and over

Number of characters possessed	Age at onset before 7 years		Age at onset 7 years and above	
	Number	Per cent	Number	Per cent
II	2	22.22	7	77.78
III	12	44.44	15	55.56
IV	22	56.41	17	43.59
V	28	70.00	12	30.00
VI	8	80.00	2	20.00

TABLE V
Paralyzed girls—Ages 7 and over

Number of characters possessed	Age at onset before 7 years		Age at onset 7 years and above	
	Number	Per cent	Number	Per cent
I	1	20.00	4	80.00
II	2	28.57	5	71.43
III	7	41.18	10	58.82
IV	18	60.00	12	40.00
V	18	72.00	7	28.00
VI	11	78.43	3	21.43

correlation between age of onset of the disease and the number of characters exhibited at the time of measurement. Thus, those children who possess a larger number of the six characters had a tendency to develop the disease at an early age.

At this point it might be of interest to determine which of the selected six characters appeared most frequently in the sortings on both groups of paralyzed children and the control sample of well boys. In a comprehensive ranking table we have listed the traits in order of their frequency in the sick and control groups (Table VI). For example, black spots were found in the highest percentage of the sick boys and girls irrespective of whether they possessed one or six of the six characters. Black spots take second place in the series of well boys and large central incisors the leading position.

From Table VI we see that the well boys do exhibit fairly high percentages for certain of the six selected characters. For example the highest is that of 52.63 per cent for large central incisor teeth. But, on the other hand the lowest percentage for any character among the afflicted group is 47.94 per cent. When, however, one compares the occurrence of the character of internal eyefolds among sick and well, it appears

TABLE VI
Percentage distribution of the six characters

Paralyzed boys 146			Paralyzed girls 124			Well boys 214		
	Num ber	Per cent		Num ber	Per cent		Num ber	Per cent
1 Black spots present	137	93 84	1 Black spots present	114	91 94	1 Large central incisors†	110	52 63
2 Large central incisors*	114	91 20	2 Large central incisors†	89	83 18	2 Black spots present	101	47 20
3 Long eyelashes	119	81 51	3 Hyperextensibility of joints pronounced	94	75 81	3 Long eyelashes	95	44 39
4 Hyperextensibility of joints pronounced	94	64 38	4 Long eyelashes	89	71 77	4 Hyperextensibility of joints pronounced	81	37 85
5 Central incisor spacing	78	53 42	5 Internal eyefolds	69	55 64	5 Central incisor spacing	78	36 45
6 Internal eyefolds	70	47 94	6 Central incisor spacing	68	54 84	6 Internal eyefolds	51	23 83

* Based on 125 cases of age 7 years and above

† Based on 107 cases of age 7 years and above

‡ Based on 209 cases of age 7 years and above

that 47 94 per cent of the former possess it, and only 23 83 per cent of the latter

A good deal of speculation has been spent over the factors which determine the distribution of the paralyzes in any given case. There has never been any rhyme or reason for the irregular and varying involvement of an arm here, a leg there, a diaphragm, or glottis. Our investigation of this point shows first that lower extremity paralysis outnumbers by a great margin any other group of muscles. This is old knowledge. Statistical analysis of paralysis location in relation to age at the time of attack, however, shows that 57 75 per cent of those children who were stricken during the first 7 years of life were paralyzed only in the lower extremities. Only 14 08 per cent of this age epoch were extensively paralyzed in trunk and limbs (general). The boys of the age epoch 7 and over, on the other hand, had a different record. In their case only 24 49 per cent suffered lower extremity paralysis whereas 32 65 per cent had general involvement. These differences are statistically significant. The relation of number of characters and age-paralysis distribution is not absolute. In the children stricken under 7 years of age there is a tendency for the possessors of five and six characters to be paralyzed in the lower extremities with much greater frequency than in other parts of the body. On the other hand, those individuals maimed after 7 years of age and who possess fewer of the six characters display a greater trend toward upper extremity and more general involvement.

The greater frequency of lower extremity paralysis has always been an unexplained and challenging phenomenon of poliomyelitis. Our figures seem to show that children afflicted at a very early age are more apt to develop lower extremity paralysis than those afflicted at an older age. If one were to theorize upon the significance of this observation, one is led to consider the fundamental biology of extremity development in quadrupeds generally. It is well known that in such forms, including man, the anterior limb buds appear first and for 2 years at least, following their appearance, take the lead in size and co-ordination of movements. Presumably the cervical enlargement of the cord parallels this growth. We do not know, however, what the relationship is during growth and development between maturing achievement and local tissue resistance to the virus attack upon the anterior horn cells. Variations in general immunity in relation to age are well known however.

The data seem to show two things. The first is that individuals possessing 5 or 6 of the specific criteria tend on the one hand to have contracted the disease at an early age. Secondly, their paralyzes involve the extremities, especially the lower. On the other hand, the children who develop their disease between 7 years and puberty more often appear to possess relatively few of the six characters. Furthermore, the paralysis of this age span is much more apt to involve arms and trunk relatively more often than is the case in the younger group. Cases which arise be-

tween puberty and full adulthood tend not only to a similar generalization of paralysis but also to increased severity of the clinical course. Thus, for example, among the 23 boys who were 15 years or over when measured in 1938 10 did not contract the disease until the 1935 epidemic at which time they were 13 or older, and at the time of the large 1931 epidemic were 9 or older. Of these, 70 per cent developed widespread paralyzes of the trunk and extremities. The majority of the members of this group did not have more than three of the selected characters. Why these boys who possessed criteria of susceptibility resisted the virus in the very active 1931 epidemic and succumbed with severe attacks in 1935 is at present unanswerable. But in view of the principle of total organismal growth and development, it may be significant.

The purpose of the foregoing study has been to show that from the morphological standpoint alone children who develop infantile paralysis are definitely different from those who resist the virus. From our point of view, human morphology is significant only insofar as it correlates with other characters of the total personality. As yet we have made no studies of other panels of the total personality of infantile paralysis patients.

The preceding paper (1) and the present one have reported a fairly large group of sick and an even larger control group of well children studied by morphological and statistical methods. As far as appears, the original observations from this clinic on morphology of infantile paralysis subjects have been largely substantiated by the analysis, and through this medium possibly some new thoughts about the nature of the disease have emerged.

In addition to the well known fact that young children are most frequently attacked, there seem to be certain peculiarities about the disease which show that the time or age factor is related to susceptibility throughout the course of growth and development. Thus, for example the differences in growth rhythm between the sick and control series is one instance of this force (1). Variations in teeth size and placement, fat contours and genital irregularities, and retardations provide other examples, while still others are found in the bony arrangements of eye zone, eye

form and folds. Finally, the retention by the susceptible group of many development criteria common to the fetus and infant such as wide inter inner canthus space, internal eye folds, separated incisors, and hyperextensibility of finger joints still further emphasize the point in the field of development. The importance of pigment irregularities, especially in the form of small black spots, is definitely the most outstanding and obscure feature. In addition to the points summarized from the first paper, the present study deals with the level of maturing achievement (age, biological time?) at the moment of disease onset, the number of the six characters present, and the location of the paralyzes.

CONCLUSIONS

- 1 The special constitutional qualities of persons susceptible to infantile paralysis are the result of genetic or adverse intrauterine influences and not the product of the disease.

- 2 Paralyzed individuals show a higher percentage of a greater number of the six characters than do the well.

- 3 There is a correlation between the number of characters a child shows and the age at which he was measured. This is true for both paralyzed and well children although the former show a larger percentage of these characters at every age level.

- 4 There is also a correlation between the age of onset of the disease and the number of characters exhibited at the time of measurement.

- 5 Both paralyzed and control groups possess one or all six characters. But in every character the stricken persons possess them in higher percentage. Furthermore, the highest percentage in any one character of the well group (52.63 per cent) is only 5.7 per cent higher than the lowest of the sick (47.94 per cent). In every case the percentage difference for a given character is significantly higher for the paralyzed group.

- 6 Individuals stricken before 7 years of age have the highest percentage of lower extremity paralysis and the least upper extremity and general involvement. Those who develop the disease after the seventh year show a high percentage of upper extremity and general paralysis.

7 Children stricken under seven years of age show (1) higher percentage of lower extremity paralysis and (2) possess a greater number of the six characters

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BIBLIOGRAPHY

- 1 Draper, G, and Dupertuis, C Wesley, The nature of the human factor in infantile paralysis I Peculiarities of growth and development. J Clin Invest, 1939, 18, 87

METABOLISM IN IDIOPATHIC STEATORRHEA. I. THE INFLUENCE OF DIETARY AND OTHER FACTORS ON LIPID AND MINERAL BALANCE

By SAMUEL H. BASSETT, E. HENRY KEUTMANN, HENRY VAN ZILE HYDE,
HELEN E. VAN ALSTINE AND ELLA RUSS

(From the Department of Medicine, School of Medicine and Dentistry, University of Rochester, and the Medical Clinic of the Strong Memorial and Rochester Municipal Hospitals, Rochester, N. Y.)

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When idiopathic steatorrhea of temperate climates and tropical sprue are compared, certain clinical and biochemical similarities are noted. Both are chronic diseases during which remissions not infrequently occur, both exhibit a peculiar fatty and fermentative type of diarrhea with bulky foul stools, anemia, sometimes resulting from a deficiency of iron, more often of the macrocytic type is common in each (1, 2, 3, 4, 5). The tongue is often sore and the papillae atrophic, when the diseases are well advanced oral ingestion of glucose causes only a slight elevation of blood sugar (3, 6, 7, 8). Reliable anatomical studies are few and diagnostic lesions have not been described in either disease (3, 8). Owing to defective absorption from the gastro-intestinal tract there is gradually progressing emaciation. Almost every sort of recognized deficiency has been described in connection with these syndromes (9). Insufficient production or absorption of the anti-anemia principal found in liver (10) seems to lead to the most characteristic deficiency of sprue (2), while hypovitaminosis D and its consequences are rather more frequent in idiopathic steatorrhea (1).

Review of the histories of patients with steatorrhea points in some cases to the origin of symptoms as celiac disease in childhood (1, 11, 8), in others the disease seems to have developed after maturity (9, 12). Apparently the relationship between celiac disease and idiopathic steatorrhea has not been questioned recently. Thaysen (8) regarded the latter as the non-tropical equivalent of sprue. English investigators, on the other hand (1, 3), have not accepted Thaysen's arguments as proof of the identity of steatorrhea and sprue. Hypotheses with respect to etiology differ somewhat (1, 2, 13), but as a general proposition it may be stated that breakdown in the absorptive

mechanism of the gastro-intestinal tract produces the clinical syndrome in each condition. Whether this malabsorption is the result of hormonal or dietary deficiencies is unknown.

Failure to recognize a specific etiology has led to diverse forms of treatment, particularly in idiopathic steatorrhea. Those who believe that a disturbance in digestion and absorption of starches may be of primary importance give special attention to correcting that defect (14, 15), those who believe that all deficiencies stem from defective absorption of fats (4, 16) find low fat diets palliative. Those who focus attention primarily on deficiencies such as avitaminosis B or D or lack of some principle occurring in liver (2, 17, 18, 19, 20) assign their favorable results to adequate replacement therapy. Still others (11, 13) are convinced that success is achieved only by the judicious combination of all methods of treatment.

In studying four patients whose clinical histories, physical status, and metabolic abnormalities characterized them as cases of idiopathic steatorrhea, evidence for or against the value of any particular therapeutic régime has been gathered while the subject was on a rigidly controlled diet. Although the individual's subjective impressions of his condition have had to be discounted in some instances, they have usually coincided with more objective data, such as the weight of the body, the weight of the stools, the amount of lipid in the stools, the degree of anemia, the vitamin A absorption and glucose tolerance curves, the concentration of protein, calcium and inorganic phosphorus in the serum and the calcium, phosphorus, and nitrogen balances. These data have also served to demonstrate the interrelation between several of the metabolic defects.

GENERAL PROCEDURE

The patients were admitted to the metabolism unit after a preliminary period of observation, during which the diagnosis was established. The diets employed were carefully weighed and fed to the subjects by a nurse, especially trained in this procedure. Once a given dietary régime was begun it was continued without variation of the menu until it was thought that the desired information had been obtained. In a few instances experimental periods were discontinued when the patient rebelled at the monotony of the routine.

Care was taken to collect the urine and feces quantitatively. The stools were weighed when passed and then pooled in periods of three, four, or six days before being analyzed. Carmine was used to mark the stools in metabolic periods of the desired length. Urine collected in twenty-four-hour periods was analyzed daily for nitrogen, aliquot portions were saved and analyzed for calcium and phosphorus.

Types of diet Eight different diets were used during the course of the investigation. Their composition is shown in Table I.¹ It will be noted that the menus in several instances were closely similar. For example, Diet I differed from Diet II only in the substitution of banana and monosaccharide to replace other forms of carbohydrate, likewise Diets VI and VII differed chiefly in the omission of skimmed milk from VII, a change necessitated by a desire to obtain a much lower intake of calcium. Supplements of butter fat and soybean phospholipid were used at times to increase the amount of lipid in the low fat diets. Repeated analyses of sample diets were made for nitrogen, fatty acids, calcium, phosphorus, and magnesium. When supplementary feedings of lipid or other relatively simple foodstuffs constituted the sole departure from the basic formula, the

¹ Casec (calcium caseinate) used in the diets was contributed by Mead Johnson and Company

TABLE I
Composition of diets
(grams per day)

Articles of food	Diet numbers							
	I	II	III	IV	V	VI	VII	VIII
Orange juice	50		1200	200	200	100	40	400
Grape juice							200	
Prunes	25							
Strawberries					100			
Apple	120			120		100	100	
Banana		750	800	100	200	650	550	600
Tomato	120					100	100	
Peas	75							
Spinach					80			
Cauliflower						70		
Onion								40
Potato				100	100	100	100	
Rice							20	
Shredded wheat	20			20		20	20	
Bread, white	62	12		122	60	120	120	
Sugar, cane	25		50	25	35	30	35	
Dextrose		15						30
Casec powder			64		16			32
Steak, tenderloin					200			100
Steak, round					150	200	300	
Liver, calves								100
Milk, skimmed			1000			700		400
Milk, whole	750	750		930	440			
Cheese, cream				40				
Cheese, American	36	36		36				
Egg	206	206		36		50		
Cream 40 per cent	80	80		80	30	170	100	
Butter	25	23		30	15	40	55	10
Mayonnaise containing egg and Wesson oil							25	
Calories (approximately)	2100	2200	2100	2300	1900	2900	2900	1500
Analyses for								
Calcium	1.35	1.33	2.38	1.46	1.04	1.092	0.235	1.090
Magnesium	0.22	0.40	0.60	0.256	0.37	0.521	0.442	0.415
Phosphorus	1.67	1.69	1.93	1.53	1.55	1.808	1.127	1.561
Nitrogen	11.46	10.74	15.59	9.85	18.50	15.74	14.12	14.16
Fatty acids	104	100	2.8	113.4	67	103.8	103.1	15.5

latter was corrected by making a separate analysis of the supplement.

METHODS

References to the methods employed in the chemical analysis of the blood and of solutions of ash of urine, feces and food have been given elsewhere (21). The absorption of vitamin A was determined for us by Dr. A. B. McCoord of the Department of Pediatrics (22).

Preliminary preparation of sample diets and collection of feces differed from methods we have used previously and requires mention.

Feces The stools for a metabolic period were collected in a large glass jar and if not frankly diarrheal were covered with water and weighed. They were converted next into a homogenous suspension by means of a mechanical stirring device and sampled while being stirred. All sampling was done by weight. The samples removed were for the determination of nitrogen, lipids, dry weight, and for ashing.

Diets A series of sample diets was prepared and divided into two sets. Those analyzed for nitrogen and minerals were liquefied by adding concentrated sulphuric acid to about one-third of the original weight and heating to the boiling point in a large pyrex beaker. The liquefied material was cooled, weighed, and sampled while being stirred rapidly. Analyses for lipids were made on diets digested in glass vessels on the steam bath after addition of 50 to 100 grams of solid potassium hydroxide. Sampling was performed as in the case of the acid digests.

Determination of lipid The wet extraction procedure described by Fowweather (23) was used with certain modifications suggested to us by Dr. R. G. Sinclair of the Department of Biochemistry.

Food Diet samples were treated with solid potassium hydroxide to approximately 20 per cent of the weight of the sample. They were digested on a steam bath in a covered flask for forty-eight hours and extracted, after acidification, three or four times with ethyl ether (24). If an emulsion formed, this was broken down by centrifuging before attempting to draw off the ether layer. The ether extracts were concentrated in a separatory funnel by drawing air through it. When the ether had been removed the residue was taken up in 40 per cent alcohol alkalized with 1 per cent potassium hydroxide and then reacidified with hydrochloric acid. The lipids were extracted with petroleum ether and transferred to a saponification flask, where after evaporation of the ether they were saponified for 30 minutes using boiling 95 per cent ethyl alcohol containing 10 per cent potassium hydroxide. This second saponification appeared necessary to ensure the complete hydrolysis of all the neutral fat. Water was added to bring the concentration of the alcohol to 40 per cent, and non-saponifiable matter was removed by extraction with petroleum ether. The soap solution was then acidified and extracted with petroleum ether. The amount of non-saponifiable matter and fatty acid was determined

by evaporation of the respective fractions of petroleum ether and weighing the residues.

Feces The technique devised by Fowweather (23) for removal of total lipid from an acidified suspension of feces was followed to the point of removal of the first ether extraction. The formation of stubborn emulsions at this juncture was obviated by centrifuging. After removing the lipids and blowing off the ether the residue was alkalized and taken up in 40 per cent ethyl alcohol. The neutral fat and non-saponifiable matter were extracted with petroleum ether. The split fat remained behind as soap and the fatty acids were extracted from these with petroleum ether after acidification. Neutral fat and non-saponifiable matter were separated by an alcoholic saponification and subsequent extraction, as in the case of food. In using this procedure, certain precautions were found necessary. These will be reported elsewhere.

CASE REPORTS

Case S. B.

This case illustrates the gradual evolution of the syndrome of idiopathic steatorrhea during a period of observation lasting ten years.

A male furniture salesman, aged 36 years born in Wisconsin but living the remainder of his life in the vicinity of Rochester, New York, entered the Rochester Municipal Hospital because of respiratory infections on August 3, 1927 and again on April 5, 1932. At the time of the second admission complaints of poor appetite, pyrosis and gaseous distention of the abdomen were mentioned but were not investigated. He had had attacks of asthma between the ages of 24 and 34 coming on in August and September but none for two years. He described his bowels as regular, the stools as normal in consistency and formed. He had always been thin, weight varying between 54 kgm. (120 lbs.) and 59 kgm. (130 lbs.). Physical examination revealed a small, pale, undernourished, chronically ill man. Height 164 cm. Weight 50.6 kgm. Blood pressure 115/70. The subcutaneous fat was sparse, buttocks flattened, with marked folding of the skin along the navel cleft. Several of the teeth were carious. Hemoglobin 80 per cent, leukocytes 8,200, urine normal, stool formed, Wasserman and Kahn reactions were negative. An x-ray of the chest was normal except for a few calcified hilar lymph nodes.

In October 1933 the patient entered another hospital with an attack of diarrhea lasting ten days and in August 1934 returned to the Municipal Hospital with the same complaint. He appeared wasted and dehydrated. Weight 42 kgm. The tongue was clean and smooth, the abdomen was distended, tympanic, and tender to pressure. Bacteriological examination of the feces and search for amebae revealed nothing of importance. There was definite macrocytic, hyperchromic anemia (significant laboratory examinations for this and subsequent admissions have been tabulated in Table II). Free hydrochloric acid (49 ml. N/10 acid per 100 ml.) was present in the fasting gastric contents. The

TABLE II
Case S B Tabulation of laboratory data

Date	Red blood cells	Hemoglobin	Leukocytes	Mean corpuscular volume	Serum calcium	Serum inorganic phosphorus	Total serum protein	A/G ratio	Fasting blood sugar	Non-protein nitrogen	Fecal lipid, dry feces	Blood pressure	Weight	
	millions	grams per cent		cu. micra	mgm. per cent	mgm. per cent	grams per cent		mgm. per cent	mgm. per cent	per cent	mm. Hg	kgm.	
Aug. 3 1927		11.6	8,200									115/70	50.6	
Apr. 6, 1932	3.5	10.9	6,800									80/55	50.8	
Aug. 15 1934	3.3	12.0	13,300	108	8.0	3.0	4.5	1.64				95/60	42.2	
Sept. 11, 1934	3.2	11.3	9,600						69				46.8	After 30 ml. liver extract (Lederle) i.m. 34 grams iron and ammonium citrate p.o.
Dec. 8, 1934	3.6	12.3	16,400		7.2	3.7			75			75/60	37.2	Loose stools
Jan. 23 1935	2.9	10.4	10,000				4.9	1.33		21		90/60	43.6	Liver extract (Lilly) p.o. equal to 600 grams liver daily for 21 days
May 16, 1935	3.26	11.7	12,500	110	6.3	2.1	4.5	1.81		33		95/65	43	Active tetany Diarrhea
June 8, 1935					6.8	2.5				25			37.2	Active tetany after parathormone. 20 units s.c. daily for 8 days
July 3, 1935					7.7	3.2					50		39.8	No more tetany Received viosterol (Squibb) 250 D 40 drops daily 12 days. Liver extract p.o. equal to 600 grams liver daily 21 days
July 20, 1935	2.9	10.0	6,500	108	8.70	3.4	5.7	2.0	73				42.8	No liver 16 days. Viosterol continued. Special diet 16 days
Aug. 21, 1935	3.0	10.0			8.7	4.7	5.5	1.9					51.8	Viosterol and special diet continued
Sept. 17 1935	2.8	11.0		125	9.5	4.0	8.3	1.84				90/65	54.0	Liver extract (Lilly) 24 ml. i.m. and liver extract (Lederle) 72 ml. i.m. 21 days
Aug. 6, 1936	4.1	14			9.0	3.7	0.6				47		61.0	Special diet—viosterol. Ferrous sulphate 11 months
July 2, 1937					6.8	2.4	4.7						43.9	Viosterol stopped Aug. 6, 1936. Diet as desired—3 months. Loose stools—3 months

no icterus Stools were soft and unformed, they were not examined for fat on this admission

Because of the anemia 30 ml of liver extract (Lederle) was given intramuscularly during seven days The reticulocytes increased from 1 per cent to 5 per cent but the red cells and hemoglobin did not increase. Subsequent administration of 34 grams of iron and ammonium citrate did not affect the anemia. A biopsy of the sternal bone marrow showed hypoplastic erythroid elements

The diarrhea subsided within 48 hours and the patient gained strength slowly He was discharged after one month weighing 45.8 kgm. The etiology of the anemia and diarrhea was not determined.

Ten days later the patient returned complaining of painful gaseous distention of the abdomen A barium enema showed the colon greatly distended and redundant, it required four to five times the usual quantity of barium suspension to fill it.

In November 1934 there was another sudden recurrence of diarrhea which led to hospitalization on December 8th

Again there was flatulence, distention, and dull shifting abdominal pain accompanied by anorexia, nausea, and vomiting The patient was profoundly emaciated, the weight was 37.6 kgm. Trousseau's sign was positive, Chvostek's negative. There was hypocalcemia and hypoproteinemia (Table II) Stools at first were fluid, brown-green, and had a very foul odor The condition remained extremely grave for three weeks Active diarrhea was controlled during the first few days by the administration of camphorated tincture of opium but the stools remained unformed, were grayish in color, and sometimes contained traces of occult blood. Fluids

were given parenterally On January 6, 1935, he had recovered sufficiently to be able to take a more liberal diet. Liver extract (Lilly) equivalent to 600 grams of whole liver was taken daily by mouth for 21 days There was little improvement, and he was discharged to the County hospital for continued care. Weight 43 kgm. The nature of his disease was still unrecognized He left the County Hospital after 10 weeks and re-entered the Municipal Hospital during a severe relapse on May 16, 1935 Besides diarrhea there was now active tetany An x-ray of the right knee joint showed marked atrophy of the bony structures Fine opacities on the cortex of each lens were noted on examination of the eyes with the slit lamp The weight declined from 43 to 37 kgm. during a period of 30 days Intramuscular parathormone, intravenous calcium gluconate, calcium lactate by mouth and 45 drops daily of a solution of viosterol in oil (Squibb 250 D) were given with some relief of tetany The stools decreased to 3 or 4 daily after a few days of rest in bed, but nausea and abdominal distention and discomfort persisted. Oral liver extract (Lilly) equivalent to 600 grams fresh liver daily was administered for 31 days There was no improvement

In July 1935 the diagnosis of idiopathic steatorrhea was made and the diet was altered radically The patient was given a diet of 1,500 calories, low in fat, relatively high in protein, and with all starchy foods replaced by monosaccharides and banana. Viosterol was continued. Within ten days crampy abdominal pain and gaseous distention, almost constant features of the illness for 18 months had disappeared. The appetite returned, and the stools gradually became formed. The caloric intake was increased slowly by the addition of more ripe banana, orange juice, cream, and butter to the diet until ap-

proximately 4,000 calories were taken daily. When return of appetite, formed stools, increase in strength, and a gain in weight of 8 kgm. indicated that convalescence was well established, it was decided to test the effect of liver extract on the anemia, until now unchanged. Accordingly during a period of 40 days the patient was given 24 ml. of concentrated liver extract (Lilly) intramuscularly followed by 72 ml. of intramuscular liver extract (Lederle). As on previous occasions the anemia was not affected.

Recovery continued and at discharge in September 1935 the weight had increased to 54.6 kgm.

The diet prescribed in the hospital was followed at home for eleven months. Ten grams of calcium lactate, 4 ml. of syrup of ferrous sulphate and 10 drops of viosterol (250 D) were taken daily. The red cells increased slowly to 4 million, and the hemoglobin to 14 grams per 100 ml. serum calcium and inorganic phosphorus remained within normal limits. Stools were partially formed but more than 40 per cent of the dry weight was lipid.

In August 1936 he stopped taking medication and began to experiment with his diet. Aside from the fact that he ate liver once or twice weekly his personal preference was permitted to dictate his choice of foods. He began to lose weight and strength on this régime and in March 1937 complained of stiffness of the hands. The stools were more frequent and were loose. The serum calcium decreased to 6.1 mgm. per cent, the inorganic phosphorus to 2.7 mgm. per cent but there was no frank tetany. He continued to become gradually worse until his admission to the metabolism unit July 1 1937.

Data obtained on this admission have been referred to in the text of this report. He left the hospital against advice on July 25 1937. Three days later the right knee became tender and swollen, followed in another day or two by a painful swelling of the right ankle. He was readmitted to the hospital on August 3 1937.

Joint involvement was present as noted above in addition several moderate sized ecchymotic spots were found on the lower legs which according to the patient's statement, were unrelated to trauma. The urine was found to be grossly bloody the stools gave a positive guaiac test for blood and blood withdrawn on venipuncture clotted only after 40 minutes. Clot retraction was poor and the clot was friable. Bleeding time was 11 minutes. Platelets were present in abundance in a smear of blood from the finger. Rumpel-Leede test for capillary fragility was negative. When as little as 0.05 ml. of normal serum was added to 2 ml. of the patient's blood a firm clot was obtained in 6 minutes. Without normal serum clotting occurred in 35 to 55 minutes. Fibrin could not be obtained from oxalated plasma by recalcification until a small amount of normal serum was added. Fibrin thus determined was 270 mgm. per 100 ml. plasma.

The picture seemed to fit Fanconi's description of purpura fulminans (hypothrombinemia) (25) more closely than that of scurvy.

He was given a diet low in fat, high in protein, and large amounts of orange juice. Ascorbic acid was given intravenously in doses of 0.2 gram daily on two successive days and on the third day 0.11 gram. Bleeding continued although the clotting time had fallen to 18 minutes. Transfusions of compatible blood in amounts of 500 ml. were given on August 8th 21st, and 27th. Following the third transfusion, bleeding from the urinary tract and from the bowel gradually diminished but did not cease altogether until September 25. Subsequent to each transfusion there was a decrease in clotting time and in the loss of blood from urine and stool. Ultimate recovery however seemed to depend upon the gradual replacement of some factor essential to the normal clotting of blood which probably was obtained from the diet.

The condition of the patient since discharge on September 27 1937 has remained unsatisfactory. Diarrhea has been infrequent as long as the low fat, low starch diet has been rigidly followed.

In February 1938 he contracted an upper respiratory infection and began to have diarrhea again. On February 24 1938, he was readmitted because of a questionable hemorrhage in the left groin and hematuria.

August 29 1935

Typical glucose tolerance after ingestion of 50 grams of glucose

Time, minutes	Fasting	30	60	90	120
Blood glucose, mgm. per cent	80	83	93	95	93

September 17 1935

*Vitamin A absorption after ingestion of 10 ml. of haliver oil**

Time, hours	Fasting	2	4	6	9	12	24
Vitamin A, units per 100 ml. plasma	13	15	21	53	44	64	30

August 30 1935

Plasma lipids

Total lipid	559 mgm. per 100 ml.
Neutral fat	159 mgm. per 100 ml.
Total fatty acid	333 mgm. per 100 ml.
Total cholesterol	159 mgm. per 100 ml.
Ester cholesterol	97 mgm. per 100 ml.
Phospholipid	176 mgm. per 100 ml.

Case J B

A woman 48 years old entered the Medical Clinic on December 2, 1932, complaining of (1) pain in the back and legs made worse by walking straining coughing or sneezing (2) a sore tongue, (3) urgency and frequency of urination, (4) swelling of the legs,

* The dose of haliver oil which was used in the vitamin A absorption test was estimated on the basis of body weight and vitamin A potency of the particular sample of oil available at the time. Each subject received approximately 7000 I.U. of vitamin A per kilogram of body weight.

(5) recurrent episodes of diarrhea in which the stools were white and frothy, (6) loss of weight of 20 pounds. She was born in South Dakota and had never lived in the tropics or in the Southern United States.

Past history revealed many years of chronic invalidism. At the age of 3 a febrile illness was followed by abdominal distention. During the subsequent 8 to 9 years abdominal paracentesis for removal of fluid was performed on a number of occasions. Tuberculous peritonitis was suspected at the time. During adolescence health was fair but at 20 years bouts of diarrhea began with large, pasty, and sometimes foamy stools. The general course has been one of exacerbation and remission since. In 1926 she was admitted to another hospital where a macrocytic type of anemia was discovered.

Physical examination Weight 50 kgm., height 161 cm., blood pressure 90/50. A small rather poorly developed thin woman with marked dorsal kyphos. Skin was dry, inelastic, and scaly over the lower trunk, with diffuse brownish pigmentation of the face and about the neck. The muscles were flabby and of poor tone. The spine was moderately tender to percussion and the ribs to pressure. The eyes, ears, and nose were not remarkable. The jaws were edentulous, and the tongue was smooth and red. The heart and lungs were normal. The abdomen showed marked gaseous distention but no fluid. The liver was palpable at the costal margin. Positive neurological findings were hyperesthesia below the level of the fifth dorsal vertebra, vibratory and position sense intact.

Urine was infected with colon bacilli but no tubercle bacilli were found in it either by direct examination or after injection into a guinea pig.

Stool was soft and unformed, 57 per cent of the dry weight was lipid.

Blood Red cells 34 million, hemoglobin 11 grams per 100 ml., leukocytes 5,500. A smear revealed many macrocytes.

Gastric analysis revealed free hydrochloric acid equivalent to 30 ml. N/10 acid per 100 ml. gastric juice after injection of histamine.

Glucose tolerance after ingestion of 50 grams of glucose

Time, minutes	Fasting	30	60	90	120
Glucose in blood, mgm per cent	76	92	87	83	78

Röntgenograms (1) Chest, slight prominence of left ventricle and aortic knuckle but otherwise normal, (2) barium enema, moderately dilated colon, (3) antero-posterior abdominal film, marked diffuse decalcification of the lumbar vertebral bodies and the pelvic bones, (4) gastro-intestinal series. Spastic pyloric sphincter, greatly increased peristaltic activity of the duodenum with delayed emptying, suggesting obstruction.

In view of the apparent duodenal obstruction and persistent complaint of burning sensations in the center of the abdomen an exploratory laparotomy was performed. The duodenum was markedly dilated. The mesentery

of the small bowel was unusually fatty and no blood vessels could be seen through it. The paraduodenal fossa was quite deep and the operator considered the possibility that prolapse of the bowel into it might have caused obstruction. No lesion, adhesion, or congenital band that could have caused obstruction was found. A mesenteric lymph node was removed for microscopic examination. The pathologist reported a mild chronic non-tuberculous lymphadenitis.

Recovery from the operation was uneventful, and the patient was discharged on March 2, 1933, somewhat improved.

She was readmitted on January 3, 1936, because of a recent fracture of the right greater trochanter, and fractured ribs following a slight fall. Other x-rays taken on this admission disclosed old infractions of the right femoral shaft and pubic bone probably the result of decalcification with pathological fractures. The dorsal spine was affected by diffuse atrophy and collapse of many of the vertebral bodies producing a profound kyphos. The picture was one of advanced osteomalacia.

The serum calcium was 80 mgm per cent, inorganic phosphorus 23 mgm per cent and total protein 58 grams per cent. Glucose tolerance was the same as previously reported.

Vitamin A absorption after 5 ml of haliver oil

Time, hours	Fasting	2	4	6	9	12	24
Vitamin A, units per 100 ml plasma	9	11	15	31	25	32	19

Free hydrochloric acid which had been present in the gastric contents on the 1932 admission was now absent after injection of 0.5 mgm. of histamine. The red blood cells numbered 3.8 million, and the hemoglobin 11.4 grams per 100 ml. The administration of 46 ml of liver extract concentrated (Lilly) by intramuscular injection during an interval of two weeks has been referred to elsewhere. It seemed to be accompanied by subjective improvement, but in the absence of a reticulocyte response, relief of anemia, change in the chemical analysis of the feces or gain in weight, we were unable to evaluate its effect. The subsequent course of the patient has been unsatisfactory. She has remained a chronic invalid and has cooperated poorly, returning only at rare intervals for advice with respect to diet and medication.

Case R. G.

A clergyman, aged 43 years, had had periodic attacks of diarrhea for 20 years. Except for a few months spent in the Southwestern United States he had always lived in the Northern States or in Canada. Six months prior to admission he became incapacitated because of loose, voluminous stools, loss of weight and strength, anorexia, nausea, flatulence, and burning sensations in the epigastrium. He described a recent attack of tetany.

Physical examination Weight 53 kgm., height 168 cm., blood pressure 98/80.

Appearance was that of moderate undernourishment. Tongue was clean, red with atrophic marginal papillae.

Abdomen was moderately distended with gas. Examination of the heart, lungs, abdominal organs, genitalia and rectum were normal. Neurological examination revealed no signs of tetany. Tendon reflexes were sluggish and elicited in the lower extremities only after reinforcement. There was no loss of vibration or position sense.

Urine was normal. Stools were very large, one passed shortly after admission weighed 900 grams, was of pale grey color soft in consistency, unformed, with a foul and sour odor. Fifty-eight per cent of the dry weight was lipid. The guaiac test for blood was negative.

Blood count. December 31 1936. Red cells 3.7 million, hemoglobin 12.1 grams per 100 ml., leukocytes 5400, mean corpuscular volume (Wintrobe) 100 cu. ml.

Blood chemistry. Serum calcium 6.7 mgm. per cent inorganic phosphorus 1.36 mgm. per cent total protein 5.7 grams per cent nonprotein nitrogen, 32 mgm. per cent.

Gastric analysis. No free HCl in fasting specimen. After 50 ml of 7 per cent alcohol free HCl was present equivalent to 23 ml. N/10 acid per 100 ml of gastric content.

Glucose tolerance after ingestion of 60 grams of glucose

Time, minutes	Fasting	30	60	90	120	180
Glucose in blood, mgm per cent	77	82	101	99	101	90

Vitamin A absorption after ingestion of 10 ml of haliver oil

Time, hours	Fasting	2	4	6	9	12	24
Vitamin A, units per 100 ml plasma	4.4	7.4	5.9	14.4	8.4	15.9	9.4

X-ray of the dorsal spine showed marked osteoporosis.

Response to therapy. Administration of intramuscular liver extract (Lilly) in connection with a high fat diet as described in the following paper (27) failed to cause improvement in the steatorrhea. There was no reticulocyte response and after a month of treatment the red blood cell count was still 3.7 million and the hemoglobin 12.4 grams per 100 ml. With ingestion of a low fat diet, containing carbohydrate mostly in the form of monosaccharide, the stools became normal in appearance and nearly normal in their content of lipid. There was some gain in weight (about 2 kgm.) on this régime but no change of note in the blood. A vitamin D concentrate was now given without change in the diet. The weight remained the same but strength improved as did the anemia and the calcium and inorganic phosphorus content of the serum. At time of discharge on June 24 1937 the red blood cell count was 4.68 million, and hemoglobin 14 grams per 100 ml.

Glucose tolerance after ingestion of 60 grams of glucose

Time, minutes	Fasting	30	60	90	120	180
Glucose in blood, mgm per cent	81	116	108	99	86	69

Vitamin A absorption after ingestion of 10 ml of haliver oil

Time, hours	Fasting	2	4	6	9	21
Vitamin A, units per 100 ml plasma	10.5	13	27	27	38	21

Since leaving the hospital the patient has taken a diet containing about 80 grams of fat, relatively high in protein and has practically excluded starchy foods from the menu. He has gained 10 kgm. in weight and has had no recurrence of the symptoms of steatorrhea.

Case P A

This patient entered the clinic on February 8 1935 at the age of 14 years. His complaints at that time were weakness, poor appetite, and failure to grow normally during a period of five years.

Physical examination. Weight 31.4 kgm., height 136 cm. blood pressure 94/58.

The principal findings were poor development, pallor, carious teeth and a large protuberant abdomen.

Urine was normal. Stools were soft, unformed, and of foul odor. 57 per cent of the dry weight was lipid. A guaiac test for blood was negative.

Blood count. Red cells 4.5 million, hemoglobin 10.5 grams per 100 ml., leukocytes 8,600. A smear showed rather small red cells poorly filled with hemoglobin.

Blood chemistry. Serum calcium 9.2 mgm per cent inorganic phosphorus 5.1 mgm. per cent, total protein 6.2 grams per cent nonprotein nitrogen 29 mgm. per cent plasma fatty acids 215 mgm. per 100 ml. phospholipid 50 mgm. per 100 ml. cholesterol 103 mgm. per 100 ml.

Glucose tolerance after ingestion of 44 grams of glucose

Time, minutes	Fasting	30	60	120	180
Glucose in blood, mgm per cent	74	77	74	78	85

Vitamin A absorption after ingestion 3 ml of haliver oil

Time, hours	Fasting	2	4	6	9	12	24
Vitamin A, units per 100 ml plasma	12	10	10	19	20	20	16

Intracutaneous tuberculin test negative.

Course. After a year on a diet low in fat high in protein and containing carbohydrate largely in the form of fruit juices and banana, the weight had increased to 45.8 kgm. and the height to 151 cm. Many of the stools were quite normal in appearance, others were soft and grey. The fecal lipid had decreased to about 35 per cent of the dry weight of the stool.

November 3 1936 he was admitted to the metabolism unit for study as described in the text. Some of the laboratory data not listed elsewhere follow.

Blood count. November 3 1936, red cells 5.1 million, hemoglobin 12.7 grams per 100 ml., red cell hematocrit 38.5 per cent mean corpuscular volume 75.3 cu. ml. mean corpuscular hemoglobin 25 mlm.

Glucose tolerance after ingestion of 46 grams of glucose

Time, minutes	Fasting	30	60	120	180
Glucose in blood, mgm per cent	97	105	102	96	95

Vitamin A absorption after ingestion of 10 cc of haliver oil

Time, hours	Fasting	2	4	6	12	24
Vitamin A, units per 100 ml plasma	11	12	42	42	13	11

The blood serum was also analyzed for calcium and inorganic phosphorus on a number of occasions, both before and after administration of liver extract and the vitamin D concentrate. Representative values were calcium 10 mgm. per cent, inorganic phosphorus 48 mgm. per cent. Neither form of medication appeared to influence these values, nor was the blood count changed from that of November 3, 1936.

The patient has continued the dietary régime instituted prior to the metabolic study and when last seen on October 14, 1937 he weighed 52 kgm. and his height had increased to 162 cm.

Effect of constant diet on fecal lipids The ingestion of a constant diet by subjects with either severe or mild steatorrhea led after an interval of several days to a fairly constant average elimination of lipid in the feces. Since the severity of the steatorrhea was apparently a measure of the disturbed absorption of fat, the effect on fat absorption of different procedures could readily be tested after the average loss of fat on a given dietary régime had been established. The fecal lipids of the juvenile patient were but little above the normal level when the intake of fat was high (Table III). In the two more severe cases the fecal loss was in excess of 50 per cent of the intake. A considerable variability in the amount of lipid excreted was noted in short metabolic periods and seems to have resulted from unavoidable errors in the demarcation of feces, which were usually soft and sometimes diarrheal in character.

In general the amount of fecal fat rose and fell paralleling the dietary fat. Appreciable changes in quantity of fecal fat could, however, be brought about by alterations in diet and medication which did not involve an increase or decrease in dietary fats. These will be discussed in their proper sequence.

Little tendency toward spontaneous exacerbations or remissions was noted during intervals of a month or more. In one case steatorrhea was increased during an acute respiratory infection.

Dietary carbohydrate and steatorrhea While one gains the impression from the literature that intolerance of starch may be as much a fault in steatorrhea as intolerance of fat, the effect of starch on fat tolerance does not appear to have been studied quantitatively. An effort in this direction was made in Case J B who was given a diet containing both fat and starch for 15 days

TABLE III
Lipid excretion in severe and mild steatorrhea
(Daily average in grams)

Diet		Period number	Number of days	Fecal weight	Total lipid	Fatty acids
Number	Fatty acids					
CASE R G						
VI	104	2-4	10	380	48.8	46.5
	104	5-9	15	459	56.0	53.7
	104	10-12	9	469	54.9	52.3
CASE P A						
VI	104	1-3	12	185	13.6	
	104	4-7	21	168	14.1	12.5
	104	8-10	18	175	13.8	12.4
CASE S B						
VIII +50 per cent	23	2	4	210	21.2	17.3
	86*	3	2	340	45.7	41.7
	23	4	4	173	21.0	18.3
	23	5	4	142	23.1	20.1
	23	6	3	152	17.9	14.8

* Includes butter fat supplement

(Diet I, Table IV). During the succeeding 18 days most of the carbohydrate of the diet was replaced by ripe banana (Diet II). There was a slight, but probably not significant, decrease in the fatty acid content of the feces, nitrogen, calcium, and phosphorus balances were not materially affected.

Some improvement might have been expected if the carbohydrate of the banana was more suited to the needs of the patient than starches (14, 15, 26). For example, less destruction of carbohydrate by intestinal fermentation might have led to better absorption and so to a higher caloric intake. A higher caloric intake from carbohydrate because of its protein sparing action might then have been reflected in increased nitrogen retention. A slightly negative nitrogen balance during the banana periods, however, did not point to a protein sparing action of this diet.

The weight increased approximately 0.7 kgm. but the well known hydrolability (25) in this disease is such that no significance can be attached to small changes in weight.

Since no correlation between steatorrhea and the quality of the carbohydrate was noticed in

TABLE IV
 Summary of metabolism studies Case J B

Period	Number of days	Diet per day							Daily medication	Daily feces					Daily balances				Body weight																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
		Number	Protein	CHO	Fatty acids	C	P	Calories (approx.)		Weight		C	P	N	Fatty acids	C	P	N																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
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1-6	15	I	72	171	104	1.85	1.87	3100	None	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams

* Includes phosphate given as medication.

† Includes calcium chloride given as medication

‡ 10 000 Vitamin D units daily intramuscularly

J B, no further specific attempt was made to change the carbohydrate fraction of the diets without altering either the amount or source of fat, and consequently less attention was given to this phase of metabolism than it apparently deserved. Later, while studying Case R. G., it became evident that some factor in addition to malabsorption of fat took part in the production of his steatorrhea. While observing his response to a low intake of fat and to vitamin D (Table V), it was found that supplements of butter fat were well tolerated. The diet to which these supplements were added (Diet VIII) contained very little starch, while the control diet (Diet VI) contained bread, rice and potatoes as well as large amounts of cream. Butter supplements eventually raising the intake of fatty acids of Diet VIII to 91 grams daily were given in Periods 38 to 41 (Table V) without increasing the steatorrhea. As the intake of fat now approached that of the control diet, the latter was substituted for Diet VIII (Period 42). The patient promptly developed abdominal distention and bulky gaseous fatty stools. Whether this response was caused entirely by the combination of fat and starch in

the diet or whether it was in part a result of the psychic upset caused by his repugnance for the particular menu in question, or to some other factor could not be established with certainty. After three days he refused further cooperation until the low fat diet was resumed. Since leaving the hospital there have been several occasions on which he has attempted to include bread, cereals, and potatoes in the diet, always with the result that he became worse. This is perhaps further evidence incriminating starchy foods in his case.

Effect of low fat diets Two very low fat diets containing most of the carbohydrate in the form of fruit juices and banana were given. Case J B received Diet III containing no meat, while Cases R. G. and S. B. were given Diet VIII containing lean meat and liver. The former diet was lower in fat, furnishing by actual analysis less than 3 grams of fatty acid daily. The energy value of Diet VIII as originally planned was too low, and after a preliminary trial it was increased by 50 per cent bringing the intake of fatty acids up to approximately 23 grams daily and calories to 2200.

Diet III was well tolerated by J B, and there

The blood serum was also analyzed for calcium and inorganic phosphorus on a number of occasions, both before and after administration of liver extract and the vitamin D concentrate. Representative values were calcium 10 mgm. per cent, inorganic phosphorus 48 mgm per cent. Neither form of medication appeared to influence these values, nor was the blood count changed from that of November 3, 1936

The patient has continued the dietary régime instituted prior to the metabolic study and when last seen on October 14, 1937 he weighed 52 kgm. and his height had increased to 162 cm.

Effect of constant diet on fecal lipids The ingestion of a constant diet by subjects with either severe or mild steatorrhea led after an interval of several days to a fairly constant average elimination of lipid in the feces. Since the severity of the steatorrhea was apparently a measure of the disturbed absorption of fat, the effect on fat absorption of different procedures could readily be tested after the average loss of fat on a given dietary regime had been established. The fecal lipids of the juvenile patient were but little above the normal level when the intake of fat was high (Table III). In the two more severe cases the fecal loss was in excess of 50 per cent of the intake. A considerable variability in the amount of lipid excreted was noted in short metabolic periods and seems to have resulted from unavoidable errors in the demarcation of feces, which were usually soft and sometimes diarrheal in character.

In general the amount of fecal fat rose and fell paralleling the dietary fat. Appreciable changes in quantity of fecal fat could, however, be brought about by alterations in diet and medication which did not involve an increase or decrease in dietary fats. These will be discussed in their proper sequence.

Little tendency toward spontaneous exacerbations or remissions was noted during intervals of a month or more. In one case steatorrhea was increased during an acute respiratory infection.

Dietary carbohydrate and steatorrhea While one gains the impression from the literature that intolerance of starch may be as much a fault in steatorrhea as intolerance of fat, the effect of starch on fat tolerance does not appear to have been studied quantitatively. An effort in this direction was made in Case J B who was given a diet containing both fat and starch for 15 days

TABLE III
Lipid excretion in severe and mild steatorrhea
(Daily average in grams)

Diet		Period number	Number of days	Fecal weight	Total lipid	Fatty acids
Number	Fatty acids					
CASE R G						
VI	104	2-4	10	380	48.8	46.5
	104	5-9	15	459	56.0	53.7
	104	10-12	9	469	54.9	52.3
CASE P A						
VI	104	1-3	12	185	13.6	
	104	4-7	21	168	14.1	12.5
	104	8-10	18	175	13.8	12.4
CASE S B						
VIII +50 per cent	23	2	4	210	21.2	17.3
	86*	3	2	340	45.7	41.7
	23	4	4	173	21.0	18.3
	23	5	4	142	23.1	20.1
	23	6	3	152	17.9	14.8

* Includes butter fat supplement

(Diet I, Table IV). During the succeeding 18 days most of the carbohydrate of the diet was replaced by ripe banana (Diet II). There was a slight, but probably not significant, decrease in the fatty acid content of the feces, nitrogen, calcium, and phosphorus balances were not materially affected.

Some improvement might have been expected if the carbohydrate of the banana was more suited to the needs of the patient than starches (14, 15, 26). For example, less destruction of carbohydrate by intestinal fermentation might have led to better absorption and so to a higher caloric intake. A higher caloric intake from carbohydrate because of its protein sparing action might then have been reflected in increased nitrogen retention. A slightly negative nitrogen balance during the banana periods, however, did not point to a protein sparing action of this diet.

The weight increased approximately 0.7 kgm but the well known hydrolability (25) in this disease is such that no significance can be attached to small changes in weight.

Since no correlation between steatorrhea and the quality of the carbohydrate was noticed in

TABLE IV
 Summary of metabolism studies Case J B

Period	Number of days	Diet per day							Daily medication	Daily feces						Daily balances			Body weight
		Number	Protein	CHO	Fatty acids	Ca	P	Calories (approx.)		Weight		C	N	Fatty acids	C	N	N		
										Moist	Dry								
1-6	16	I	72	171	104	1.85	1.57	3100	None	grams 123.8	grams 34.3	grams 1.25	grams 0.48	grams 1.37	grams 17.5	grams +0.016	grams +0.16	grams +0.51	kgs. 45.05
7-11	16	II	67	225	100	1.83	1.56	2200	None	167.2	40.1	1.23	0.73	1.56	15.0	-0.09	+0.06	-0.14	46.53
12-18	16	I	73	171	104	1.85	1.57	3100	None	153.2	52.3	1.23	0.57	1.23	14.8	+0.04	+0.04	+0.16	44.53
19-20	12	III	97	407	2.5	2.28	1.83	2100	None	113.2	25.4	2.47	1.86	1.48	1.1	-0.20	-0.03	-0.70	46.80
21	3	IV	62	223	113.4	1.46	1.53	2300	None	123.4	29.5	2.20	1.38	1.28	8.6	-0.80	-0.40	-0.71	47.10
22	3	IV	62	223	113.4	1.46	1.53	2300	None	55.0	22.3	1.09	0.60	0.65	11.3	+0.33	+0.28	+1.06	45.90
23	3	IV	62	223	113.4	1.46	2.81	2300	Na glycerophosphate 15 grams	255	48.7	2.22	1.67	1.49	24.5	-0.90	-0.18	+0.03	47.47
24	3	IV	62	223	113.4	2.32†	1.53	2300	HCl 253 ml. w/10. CaCl ₂ 2.19 grams	256	42.6	2.13	1.11	1.43	21.9	+0.14	-0.51	+0.53	47.30
25-29	12	IV	62	223	113.4	2.40†	2.46†	2300	Na glycerophosphate 9 grams, CaCl ₂ 3.35 grams	341	21.8	3.45	1.71	1.21	29.5	-0.13	-0.004	+0.23	47.37
30	3	III	97	407	2.5	2.28	1.83	2100	None	212.7	41.4	2.91	1.57	1.78	9.5	-0.56	-0.04	+1.63	47.66
31	3	III	97	407	2.5	2.28	1.83	2100	None	172	29	2.13	1.35	1.63	1.3	+0.19	+0.05	-0.23	47.60
32	3	III	97	407	2.5	2.28	1.83	2100	Beef bile 3.3 grams	223	32.8	2.30	1.35	1.80	1.2	-0.11	-0.13	-0.96	47.36
33	3	IV	62	223	113.4	1.46	1.53	2300	Beef bile 1.6 grams	418.5	56.7	3.45	1.30	2.74	22.9	-1.06	-0.55	-3.23	47.00
34	3	IV	62	223	113.4	1.46	1.53	2300	Beef bile 1.25 grams	453.9	37	1.57	0.63	1.66	14.4	+0.13	+0.06	-0.73	47.24
Observation discontinued for 3 days																			
35	3	V	115	183	67	1.04	1.55	1000	Liver extract 5 ml.	167.2	22.5	1.57	0.85	1.23	11.3	-0.44	-0.23	+0.74	47.73
36	3	V	115	183	67	1.04	1.54	1000	Liver extract 5 ml.	54	31.3	0.58	0.33	1.11	6.4	+0.03	+0.01	+0.91	48.09

* Includes phosphate given as medication.

† Includes calcium chloride given as medication

‡ 10 000 Vitamin D units daily intramuscularly

J B, no further specific attempt was made to change the carbohydrate fraction of the diets without altering either the amount or source of fat, and consequently less attention was given to this phase of metabolism than it apparently deserved. Later while studying Case R. G., it became evident that some factor in addition to mal absorption of fat took part in the production of his steatorrhea. While observing his response to a low intake of fat and to vitamin D (Table V), it was found that supplements of butter fat were well tolerated. The diet to which these supplements were added (Diet VIII) contained very little starch while the control diet (Diet VI) contained bread, rice, and potatoes as well as large amounts of cream. Butter supplements eventually raising the intake of fatty acids of Diet VIII to 91 grams daily were given in Periods 38 to 41 (Table V) without increasing the steatorrhea. As the intake of fat now approached that of the control diet, the latter was substituted for Diet VIII (Period 42). The patient promptly developed abdominal distention and bulky gaseous fatty stools. Whether this response was caused entirely by the combination of fat and starch in

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Effect of low fat diets Two very low fat diets containing most of the carbohydrate in the form of fruit juices and banana were given. Case J B received Diet III containing no meat, while Cases R. G. and S. B. were given Diet VIII containing lean meat and liver. The former diet was lower in fat, furnishing by actual analysis less than 3 grams of fatty acid daily. The energy value of Diet VIII as originally planned was too low, and after a preliminary trial it was increased by 50 per cent bringing the intake of fatty acids up to approximately 23 grams daily and calories to 2200.

Diet III was well tolerated by J B., and there

was almost immediate improvement in the stools, which changed from soft, pale, greasy, and unformed to normal color and consistency (Table IV, Periods 16 to 19). Complaints of bloating and anorexia were no longer mentioned. Nitrogen balances were slightly positive during this time. There was a slight loss of calcium and

phosphorus from the body in spite of a higher intake of these elements. The concentration of serum calcium increased and the inorganic phosphorus decreased. The changes in the blood have been discussed in more detail in another paper (27).

Fecal analysis showed a marked decrease in

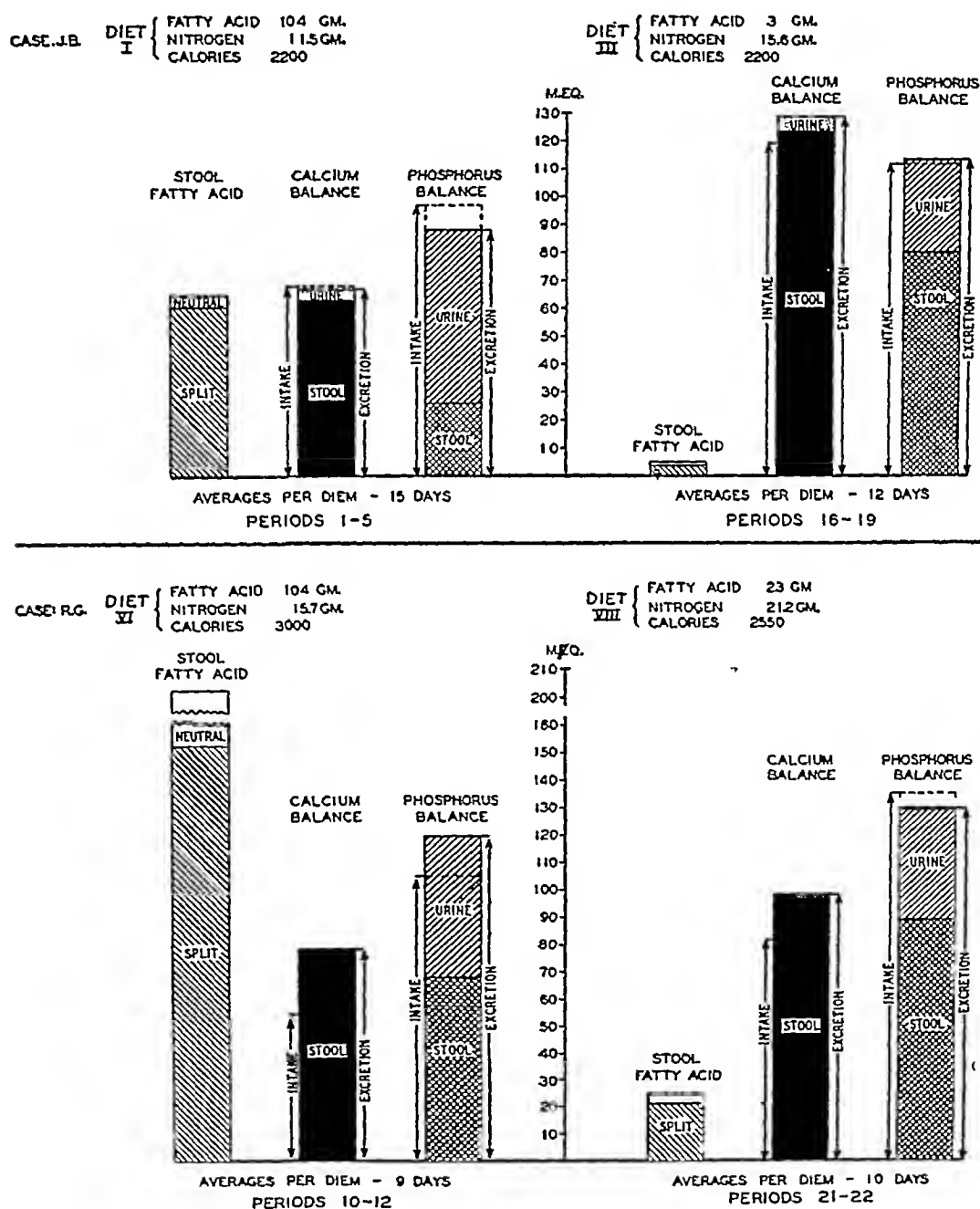


FIG. 1 CALCIUM-PHOSPHORUS BALANCES OF CASES J. B. AND R. G. ON HIGH AND LOW FAT DIET

the amount of lipid and the moist and dry weights also decreased. This form of diet abolished the steatorrhea and with it many of the more unpleasant symptoms. The comparatively short time allotted to this study did not permit deduction with regard to ultimate effects. One may safely conclude, on the basis of calcium and phosphorus balances that steatorrhea was not the only factor leading to loss of these elements from the body (Figure 1).

The effect of the second type of low fat diet on R. G. was equally satisfactory. Within 48 hours the stools became formed, abdominal discomfort largely disappeared and the fecal lipids decreased from about 49 to 12 grams a day (Table V). As previously stated, R. G. was quite tolerant of supplementary fats added to this diet after he had

received vitamin D. Case S. B., on the other hand, exhibited less initial tolerance for Diet VIII and reacted unfavorably when butter supplements were added to it (Table VI). While ingesting 23 grams of fatty acids he excreted 17 grams and after increasing the intake to 86 grams by addition of butter he excreted 42 grams. Obviously, a greater amount of fat was retained at the higher level of intake but the patient was subjectively worse, the stools increased in weight and became soft. In summary, it may be stated that low intakes of fatty acid caused a prompt decrease in the fecal lipid and subjective improvement in three patients, but did not prevent loss of calcium from the body. When fat was practically excluded from the diet (Case J. B.) fecal lipid was still less than the amount ingested. The evidence

TABLE V
Case R. G.

Period	Number of days	Diet per day				Daily medication	Fecal weight daily		Fecal lipid daily		
		Number	Fatty acids	Calories (approx.)	Supplements		Moist	Dry	Total	Split fatty acids	Neutral fat
2-4	10	VI	grams				grams	grams	grams	grams	grams
20	3	VIII*	104	2900	None	None	380	83	48.8	35.3	11.2
37	6	VIII*	23.2	2200	None	None	169	46	11.7	8.7	1.1
		VIII*	23.2	2200	None	Vitamin D	80.5	20.6	5.8	3.9	0.5
38	4	VIII*	68.2†	2700	Butter	225 000 I U	138	37	13.5	10.1	1.2
39	4	VIII*	68.2†	2700	50 grams Butter	225 000 I U	97	25	8.2	5.6	0.9
40	4	VIII*	68.2†	2700	50 grams Butter	225 000 I U	123.5	25	9.6	6.7	0.8
41	4	VIII*	90.7	2900	50 grams Butter	225 000 I U	88	17.4	8.0	5.8	0.6
42	3	VI	104	2900	75 grams None	225 000 I U	391	65.2	26.8	21.8	2.4

* Diet VIII increased by 50 per cent

† Includes fatty acids of the butter supplement

TABLE VI
Effect of increased intake of fat on calcium and phosphorus balance in Case S. B.

Period	Number of days	Diet per day			Daily feces							Daily balances	
		Number	Fatty acids	Supplement	Moist	Dry	Total fatty acids	Split fatty acids	Mg	Ca	P	Ca	P
2	4	VIII*	grams		grams	grams	grams	grams	grams	grams	grams	grams	grams
3	2	VIII*	23.2	None	210	58.3	17.3	12.7	0.423	2.26	1.70	-0.64	+0.13
		VIII*	86.2†	Butter	340	91.1	41.6	33.8	0.514	2.56	1.51	-0.93	+0.20
				70 grams									

* Diet increased 50 per cent

† Includes fatty acids of butter supplement

is, therefore, against excretion of lipid into the bowel as an important factor in the mechanism of production of idiopathic steatorrhea.

Effect of oral administration of sodium glycerophosphate Verzar and Laszt (28) have suggested that formation of glycerophosphate in the intestine may be necessary for phosphorylation and absorption of fatty acids. Macrae and Morris (29), on the other hand, thought that the increased acidity of the small intestine after administration of sodium acid phosphate was responsible for better absorption of fats and minerals. A careful study of Verzar's experimental results shows that the effects he obtained may well have been caused by changes in the reaction within the intestinal loop. At pH's of 7 or greater there was little absorption of fatty acid, while at a lower pH the absorption improved. In this respect at least they confirm the results obtained by Macrae and Morris.

The administration of sodium glycerophosphate failed to improve Case J B's steatorrhea, on the contrary it was made worse (Period 22, Table IV). The diet in this instance did not conform to the original control, since the patient refused it. A similar diet (IV) was substituted in its place. In periods (20 and 21) serving as controls, the fecal lipids were surprisingly low, even though the intake of fatty acids was slightly higher than before. This moderate decrease of fecal fatty acids may have been owing to failure to develop immediately the characteristic rapid passage of intestinal contents or to somewhat better absorption of the mixture of fatty acids included in this diet.

Administration of 12 grams of sodium glycerophosphate daily for the next three days (Period 22) was followed by a sharp fall in the serum calcium, active tetany, soft stools, a great increase in fecal lipid, and markedly negative calcium and phosphorus balances. Glycerophosphate was stopped until the tetany could be brought under control. This required about four days. A single intravenous injection of 1 gram of calcium gluconate relieved the more acute symptoms. One liter of N/10 hydrochloric acid was given by mouth over a period of 3 days with the intention of counteracting the effect of the extra base given with the alkaline phosphate. Calcium chloride

solution by mouth was started on the last day of Period 23. In Period 24 the signs of tetany had disappeared and glycerophosphate was again given in amounts of 9 grams daily, together with calcium chloride as indicated in Table IV. The extra calcium was sufficient to have combined with all the extra phosphorus as tricalcium phosphate. The phosphate solution was given with meals, the calcium chloride about two hours after each meal. On this régime the serum calcium remained about one milligram per cent higher than the level at which the patient had tetany. The feces continued to be soft and unformed. Fecal weight increased as did the quantity of fecal lipid. The latter reached its maximum value in these periods. Even at the much higher levels of calcium and phosphorus intake, consistently positive balances of these elements were not obtained.

Ox bile The conflicting opinions on the value of bile salts in steatorrhea have been discussed by Macrae and Morris (29). A purified preparation of dried ox bile³ was given to Case J B in an attempt to determine whether it would have any effect on intestinal absorption. The administration was begun in Period 32 (Table IV) while the low fat diet was being ingested. Four grams were given on the first and second days of the period. Abdominal distress was noted after the first few doses and the stools became liquid. The dose of bile for the third and last day of this period was reduced to 2 grams. Complaints of nausea and of "burning like a fever in the stomach" persisted. In Period 33, the dose of bile was further reduced to 1.5 grams a day and the diet was changed to one high in fat (Diet IV). During this and the subsequent period on the same régime, the patient was quite ill and complained bitterly of the diet and of the medication. The stools continued to be loose and the fecal fatty acids were well above the previous control periods on Diet IV. Average nitrogen, calcium, and phosphorus balances were negative (Table IV). There was no loss of weight.

All metabolic observations were omitted for the five days immediately following Period 34, and the patient was allowed to make her own selection of foods. Diet V was evolved as a result. It

³ Purified ox bile was contributed by Burroughs Wellcome and Company.

was higher in protein and contained considerably less fat than the control diets. Much of the protein was derived from meat. Unfortunately, no control observations were made on this diet. Its effect has been taken up in connection with the discussion of the use of liver extract (27).

An apparent interrelation between the amount of fecal alkaline earth and fatty acid. Certain procedures causing an increase in Case J. B.'s fecal calcium were associated with an increase in the amount of split fatty acids eliminated in the feces (see Table IV). The calcium content of the feces was considerably increased by (1) administration of a solution of sodium glycerophosphate in Period 22, (2) administration of hydrochloric acid and calcium chloride in Period 23 and (3) administration of both calcium chloride and sodium glycerophosphate in Periods 24 to 29.

Undesirable side effects consisting of tetany and loose stools were encountered in Periods 22 and 23. Loose stools continued to occur in Periods 24 to 29 but there was no tetany. There was a marked increase in the amount of fatty acid eliminated in the stools of all these periods. The exclusion of tetany as a cause of the increase in steatorrhea seems justifiable on the ground that the syndrome occurred only in Periods 22 and 23. Although the laxative effect of the salts continued this never amounted to a true diarrhea, such as occurred in the bile periods 32 to 34. Frequent watery stools in the latter as well as the passage of carmine in six and one half hours indicated an acceleration in the rate of passage of the contents of the intestine which considerably exceeded that of other periods. Small, frequent, watery stools persisted until bile was discontinued at the end of the second day of Period 34. Then diarrhea stopped. The calcium content of the feces of Period 33 was much higher than in Period 34 and probably represented a lag from the higher intake and fecal excretion produced by Diet III. That the lag was not an exclusively diarrheal effect is evident from the fact that it occurred in other transition periods in which there was no diarrhea. The much higher fatty acid content of the feces of Period 33 over that of 34 becomes explicable if one postulates a decrease in the length of the absorptive period plus an increase in the

concentration of calcium ions in the gut which hindered absorption through formation of insoluble soaps. The effect of diarrhea alone on the absorption of fats of Diet IV seems better exemplified by Period 34. The irritative effects of bile in this period when combined with a lower calcium intake and a smaller excretion of fecal calcium did not lead to such marked steatorrhea. Moreover, the irritation produced by bile seems, both on clinical grounds and from the weight of the feces, to have been much greater than that of the salt solutions given in Periods 22 to 29. This is likewise against the supposition that salt effects were entirely a matter of acceleration of the rate of passage of the ingesta through the small intestine.

From the experiments cited it is evident that administration of an alkaline phosphate, a calcium salt, or a diet having a high calcium and phosphorus content, increased the amounts of these elements found in the feces. The combination of a high output of fecal calcium and phosphorus with a high intake of fatty acid accentuated the steatorrhea. Both calcium chloride, and sodium glycerophosphate might be expected to make the content of the intestine more alkaline. The former because chloride is rapidly and almost completely absorbed leaving calcium behind (30), the latter because of its alkaline buffer effect.⁴ Conditions favorable to the formation of insoluble soaps and phosphates would thus be produced. Period 22, which was the least complicated of the group, may be taken as an example. Sodium glycerophosphate by increasing the alkalinity of the small bowel could have caused precipitation of the calcium of the food and from the intestinal secretions as phosphate and quite possibly as carboxate. A further reaction between fatty acids and calcium ions or salts tended to place additional restraints on the already defective absorption of fatty acids. Four factors seem to have acted together to increase steatorrhea.

(1) A supply of fatty acids from a dietary source undergoing abnormally slow absorption.

(2) An abnormally alkaline reaction of the contents of the intestine.

⁴ The pH of a 1/10 solution of sodium glycerophosphate was found to be 8.8.

(3) An unusually high concentration of calcium ions or salts

(4) Some degree of acceleration of the rate at which the chyme passed through the small bowel

Effect of a low intake of calcium on steatorrhea
Case P A showed a rather striking interrelationship between fecal calcium, magnesium, phosphorus, and split fatty acids. In his case a decrease in the sum of milliequivalents of calcium plus magnesium was associated with a comparable decrease in the amount of fatty acids in the stool. On the other hand, there appeared to be an inverse relationship between fecal phosphorus and fatty acid.

Table VII shows that when the intake of fatty acids was constant and when the amount of calcium, magnesium, and phosphorus was known, the amounts of split fecal fatty acids could be predicted quite accurately.⁵ This held true not only in the high fat-normal calcium and high fat-low calcium diets but also in the periods when the calcium and phosphorus content of the feces was diminished by the administration of a vitamin D concentrate. While vitamin D caused more complete absorption of both calcium and phosphorus, the effect on fecal phosphorus was if anything greater than the effect on fecal calcium, and there remained in the feces as much calcium and magnesium as before which was free to combine with fatty acids. There was, therefore, no decrease in fecal split fat.

In estimating fatty acids in milliequivalents, the average molecular weight of the fatty acids was taken as 270. The base combining power of phosphorus was assumed to have been 1.8 times

⁵ For example, using the data recorded in Table VII the predicted decrease of fatty acids in feces during low calcium periods was equivalent to

$$\begin{array}{rcl} (\text{Ca} + \text{Mg of control}) - (\text{Ca} + \text{Mg of low calcium period}) & & \\ 71.4 & - & 34.9 \\ \hline & & 36.5 \\ \text{-- Decrease in phosphorus} & & \\ & & 14.3 = 22.2 \text{ m eq} \\ \text{Observed decrease} & = & 23.3 \text{ m eq} \end{array}$$

The predicted decrease of fatty acids in feces during vitamin D periods was equivalent to

$$\begin{array}{rcl} (\text{Ca} + \text{Mg of control}) - (\text{Ca} + \text{Mg vitamin of D periods}) & & \\ 71.4 & - & 50.5 \\ \hline & & 20.9 \\ \text{-- Decrease in phosphorus} & & \\ & & 20.7 = 0.2 \text{ m eq} \\ \text{Observed decrease} & = & 3.3 \text{ m eq} \end{array}$$

TABLE VII

Case P A Relationship between fecal calcium, magnesium, phosphorus, and split fatty acids

		Control periods 17 to 19 (15 days)	Low calcium periods 15 to 16 (9 days)	Vitamin D periods 22 to 25 (16 days)
Intake		grams per day	grams per day	grams per day
	Fatty acid	104.0	105.0	104.0
	Calcium	1.092	0.235	1.092
Feces	Phosphorus	1.808	1.127	1.808
		m.eq per day	m.eq per day	m.eq per day
	Split fatty acid	41.3	18.0	38.0
Feces	Phosphorus	35.7	21.4	15.1
	Total	77.0	39.4	53.1
Feces	Calcium	52.1	18.4	35.0
	Magnesium	19.3	16.5	15.5
	Total	71.4	34.9	50.5

the number of millimols of phosphorus, as it would have been at the pH of the blood. These values have been used arbitrarily in Table VII and in graphs in which fatty acids and phosphorus have been expressed in milliequivalents. No data were obtained on the nature of the fecal phosphates, and there is no justification for the choice of a base combining power for phosphorus of 1.8, other than the fact that the sum of milliequivalents of fatty plus phosphoric acids so calculated approximately balanced the sum of milliequivalents of calcium plus magnesium in the feces of Case P A. over a long period of time and under quite different experimental conditions. Nearly the same results would have been obtained had all of the fecal phosphorus been considered to be present as dicalcium phosphate.

The stools of R. G. showed changes qualitatively similar to those of P. A. (Figure 2), but there was usually no very close agreement between the sum of alkaline earths on the one hand and phosphorus plus fatty acids on the other. With the change from normal to low calcium diet there was a greater reduction in the amount of split fatty acids excreted in the feces than was predictable from the decrease in alkaline earths. Lack of a good correlation is not surprising when the severity of disturbance of fat absorption and the different rates at which the intestinal contents traversed the bowel are considered. Diarrhea introduced a particularly erratic variable when this

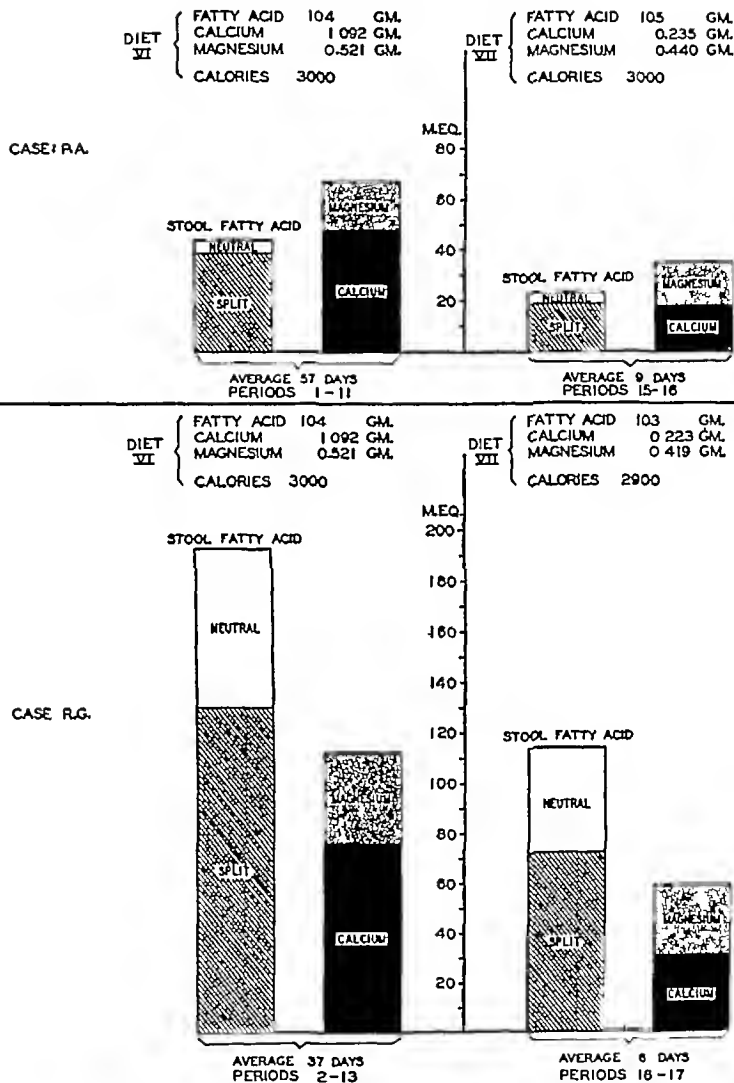


FIG. 2. DECREASE IN STEATORRHEA ACCOMPANYING THE INGESTION OF A LOW CALCIUM DIET BY P. A. AND R. G.

patient was on the high fat regime and probably prevented the attainment of a state of equilibrium in the intestine. It was usually necessary to collect the stools for from six to ten days to obtain a reliable estimate of the daily excretion of fatty acids.

The time required for passage of carmine markers was followed with considerable care. While taking Diet VII, the average rate of passage of carmine was 16 hours, the same average time as in Periods 2 to 13 which have been chosen for comparison (Figure 2). This is evidence against the assumption that the fats of Diet VII were more completely absorbed because the absorptive period was longer. If lengthening of the absorptive period is not to be offered as one of the reasons for the better absorption of fats of the low calcium diet, the most probable alternative assumption would be that at a normal level of dietary calcium, fat absorption was impeded by formation of insoluble soaps.

Low calcium-high fat diets and calcium balance Both of the subjects ingesting the high fat-low calcium diet (VII) were in negative calcium balance. The fecal lipids of the mild juvenile case of steatorrhea (P A) were brought within the normal range by this diet, but the change from positive to negative calcium and phosphorus balances was highly undesirable. In the long run, injury to the skeletal system might be expected on such a regime, which would more than offset any improvement derived from more complete absorption of fatty acids.

Interestingly enough the loss of calcium by the severe adult steatorrhea, R G, was almost the same on both normal and low calcium intakes. Representative effects of two diets containing the same amount of fat but providing normal and low

levels of calcium respectively are given in Table VIII. Total failure of calcium absorption may be offered as an explanation for R G's loss of equally large amounts of calcium at the two levels of intake. As the urine was nearly devoid of calcium, the only important avenue of excretion was the intestine. In order to have a nearly constant negative balance, one need only assume that the feces contained all of the calcium of the food plus a fairly constant daily increment derived from secretions entering the intestine.

High fat intake and calcium balance A survey of the data obtained from Subject R. G. shows that calcium loss was more pronounced when he ingested the high fat diet (VI) (Figure 1 and Table VIII). The inference is that a high intake of fat increased the excretion of calcium in the feces. The evidence must be accepted with reservation, for at the low level of dietary fat he had a higher intake of both calcium and phosphorus and no diarrhea. More direct evidence of a relation between higher intakes of fat and increased excretion of fecal calcium was obtained from another subject (S B). It was possible to supplement his low fat diet (VIII) with butter without producing diarrhea (Table VI). Under the conditions of the experiment, calcium, split fatty acids, and neutral fat in the feces increased, the first two appearing in practically equivalent amounts. The fecal excretion of phosphorus decreased slightly. The net effect was a definite increase in the loss of calcium from the body and a small retention of phosphorus. While diarrhea does not seem to have been a cause of the greater loss of calcium, the results are still open to question since the experimental period was very short.

Supplementary feeding of phospholipid Phos-

TABLE VIII
Effect of high and low calcium diets on calcium balance in Cases P A and R. G

Diet number	Diet per day			Case P A				Case R. G			
	Fatty acids	Ca	P	Periods	Number of days	Daily balances		Periods	Number of days	Daily balances	
						Ca	P			Ca	P
	<i>grams</i>	<i>grams</i>	<i>grams</i>			<i>grams</i>	<i>grams</i>			<i>grams</i>	<i>grams</i>
VI	104	1 092	1 808	8-10	18	+0 140	+0 092	10-12	9	-0 477	-0 248
VII	105	0 235	1 127	12-13	6	-0 283	-0 133	15-17	9	-0 449	-0 341
VIII	23 2	1 635	2.342					32-33	8	-0 020	+0 119

pholipid was given to one subject (R. G.) and was well tolerated. The supplements were superimposed on a low fat low starch diet (Diet VIII, Table IX). As this individual was apparently made worse by starchy food, the low starch intake may have been the major factor in his tolerance of this form of lipid. Another possibility is that the patient with steatorrhea may be able to absorb phospholipid more readily than fatty acid derived from neutral fat. In order to test this assumption he was given a commercial preparation made from soybean, which was stated to be pure lecithin but was found to be a mixture of lecithin, cephalin and perhaps phosphatide.*

The supplementary lipid was given at first mixed with shredded coconut and chocolate syrup to disguise a rather unpleasant flavor. Later the coconut was omitted. During the ingestion of these supplements some difficulty was experienced in fractionating the fecal lipids, and only the total lipid appears to have been determined with sufficient accuracy to be of significance. The total amount of lipid excreted in the feces was about doubled, while the total intake of fatty acids increased about threefold. Nitrogen retention was greater. There was consistent retention of considerable amounts of phosphorus, in spite of a negative calcium balance. The patient gained in weight. The net gain of nitrogen was 85 grams of phosphorus 111 grams while calcium was lost to the extent of 4.26 grams. Phosphorus available for metabolism was thus the 111 grams retained from the food plus about 1.9 grams de-

rived from bone or 13 grams in all. This gave an N/P ratio of 6.5, therefore, more phosphorus was retained than could have been deposited with protein. We believe this is evidence that phospholipid was retained in the body.

Fecal nitrogen. The excretion of nitrogen in the feces of patients with celiac disease and idiopathic steatorrhea has often been observed to be higher than normal (8, 25, 29, 31, 32, 33, 34), but never attains the high levels observed in pancreatic steatorrhea. This has been considered by Thaysen (8) to be caused by factors other than defective digestion and absorption of proteins. Some data from three of our cases is given in Table X.

The fecal nitrogen of J. B. was consistently normal when the protein intake varied between 62 and 115 grams daily. There was no correlation demonstrable between fecal nitrogen and fecal lipid. The nitrogen of the stools was found to be slightly higher than normal only in those periods of diarrhea which were induced by administration of ox bile. In the case of R. G., there was rather good correlation between fecal nitrogen, lipid and water. The physical effects of his failure to absorb fatty acids and his constant tendency toward diarrhea when taking Diets VI and VII seem sufficient explanation for the high fecal nitrogen. His response to administration of vitamin D while taking Diet VIII was quite definite (Period 36 to 40). Fecal nitrogen and water both decreased, the latter to an entirely normal value. Nevertheless his reaction to an unfavorable diet was not altered materially. When he was shifted back to the irritative type

* Personal communication from W. R. Bloor

TABLE IX
Metabolism of phospholipid supplements by Case R. G.

Period	Num- ber of days	Diet per day						Sources and amounts of supplementary fatty acids daily			Daily feces		Daily balances			Body weight
		Num- ber	F.A.	Ca	P	N	Calories (approx.)	Phospho- lipid	Coco- nut	Choc- olate	Weight	Total lipid	Ca	P	N	
21-22	10	VIII	grams 23.2	grams 1.64	grams 2.34	grams 21.24	2200	grams None	grams None	grams None	grams 155	grams 8.8	grams -0.323	grams +0.107	grams +1.64	grams 50.85 51.08
23	6	VIII	67.4	1.79	3.89	21.74	2800	30.7	12.1	1.4	164	14.8	-0.072	+0.807	+3.03	52.55
24-25	10	VIII	67.4	1.79	3.89	21.74	2800	30.7	12.1	1.4	213	16.1	-0.191	+0.241	+2.55	53.17
26	4	VIII	67.4	1.79	3.89	21.74	2800	30.7	12.1	1.4	199	15.0	-0.211	+0.035	+2.02	53.47
27	4	VIII	67.6	1.82	4.55	22.25	2800	43.0		1.4	220	13.5	-0.181	+0.142	+2.28	53.87
28	4	VIII	67.6	1.82	4.55	22.25	2800	43.0		1.4	194	11.7	+0.181	+0.314	+2.27	54.24
29	6	VIII	67.6	1.82	4.55	22.25	2800	43.0		1.4	192	12.2	-0.069	+0.318	+2.54	54.37

TABLE X
Fecal nitrogen, total lipid, and water

Case J B						Case R. G						Case P A					
Period	Num-ber of days	Diet num-ber	Daily feces			Period	Num-ber of days	Diet num-ber	Daily feces			Period	Num-ber of days	Diet num-ber	Daily feces		
			N	Lipid	Water				N	Lipid	Water				N	Lipid	Water
			grams	grams	grams				grams	grams	grams				grams	grams	grams
2-5	12	I	1 30	19 9	95 4	2-4	10	VI	3 05	48 8	297	4-7	21	VI	2 34	14 1	131
7-11	15	II	1 59	17 2	157	5-9	15	VI	3 68	56	355	8-10	18	VI	2 34	13 8	135
16-19	12	III	1 48	2 1	89 8	14-15	6	VII	4 40	52 4	472	14-16	12	VII	1 97	8 9	82
26-29*	12	IV	1 31	30 9	188 8	16-17	6	VII	3 57	32 7	395	22-25†	16	VI	2 26	14 9	98
31	3	III	1 63	2 1	142 5	21-22	10	VIII	2 25	8 8	112	26-27‡	8	VI	2 07	14 8	93
33-34†	6	IV	2 21	20 2	415	36-37‡	10	VIII	1 62	7 6	93						
						39-40‡	8	VIII	1 37	9 2	86						
						42‡	3	VI	2 95	26 8	325						

* Sodium glycerophosphate 9 grams, calcium chloride 5.38 grams daily

† Ox bile (See text)

‡ Vitamin D concentrate 225,000 I U daily

§ Vitamin D concentrate 100,000 I U daily

of diet (VI, Period 42) the excretion of nitrogen, lipid, and water immediately increased

Case P A's excretion of fecal nitrogen was consistently rather high, but neither diet nor medication as employed in his case produced any radical change in the composition of the feces

From the data at hand, it may be surmised that the quantity of fecal nitrogen is related to the physical properties of the feces and to diarrhea. Irritative diets produce diarrhea and, perhaps, as suggested by McCrudden and Fales (33), increase secretion of nitrogenous material (secretions from intestinal glands, diapedesis of leukocytes, etc.) into the lumen of the bowel. When these effects were not prominent, as in Cases J B and P A, there was little alteration of the fecal nitrogen from the normal, when irritative effects were prominent definite increases in fecal nitrogen were found.

COMMENT

Features common to all of the cases studied were mild anemia, flat glucose tolerance and vitamin A absorption curves, an excess of split fatty acids in the feces when a diet high in fat was ingested, and vitamin D deficiency. One subject developed a hemorrhagic disease associated with defective coagulation of the blood. In general the metabolic abnormalities correspond to those described by Fanconi (25), Lehndorff and Mautner (32), Macrae and Morris (29), and Parsons (11) in celiac disease and by Linder and Harris

(35), Bennett *et al* (1), Bauer and Marble (17), Thaysen (8), and Brull (36) in idiopathic steatorrhea of adults.

Response to dietary fat There were marked individual differences in the behavior of the four patients, but the average daily amount of fecal lipid was remarkably uniform for a given subject on a constant regime. The two more severe cases lost upwards of 50 per cent of the ingested fatty acids in the feces during periods of high fat intake. In the mildest case the loss amounted to only about 11 per cent of the intake. When the dietary fat was increased, there was a higher percentage of absorption, but a high intake frequently led to episodes of diarrhea, anorexia, and loss of weight. The advantage gained by greater absorption of fat was more than offset by loss of inorganic salts, water, and probably carbohydrate in the feces.

Low intake of fat Steatorrhea was always controlled when sufficiently stringent limitations were imposed on the intake of fat. There was no evidence to suggest that idiopathic steatorrhea resulted from an abnormally high excretion of lipid into the bowel.

Dietary carbohydrate The nature of the dietary carbohydrate did not affect the steatorrhea of one of the subjects. No improvement resulted from substituting monosaccharide for starch. In another individual the evidence suggested that an increase in fatty diarrhea accompanied the ingestion of starches. This effect was presumably

one of greater irritability of the bowel caused by fermentation Miller (16) has suggested that granules of starch become coated with fatty acids and are thus protected from the action of amylase. Bacteria within the masses then ferment the starch and produce gas and irritating split products

Alkaline earths and fecal lipid It has been pointed out that the quantity of fecal split fat was increased when the amount of alkaline earths provided by the diet was increased or when they were precipitated in the intestine by rendering the contents of the gut more alkaline. Reduction of the intake of alkaline earths while the intake of fatty acids was maintained at a constant level diminished the loss of split fat in the feces. When a higher intake of fat was given while the alkaline earths and phosphorus were kept at a constant level the loss of calcium in the feces increased. It is obvious then that there is a certain degree of interdependence between the presence of alkaline earths and fat in the feces. Such a relationship may well provide the elements of a vicious circle and accelerate the decalcification of the bones of an adult, whose diet is deficient in vitamin D.

Avitaminosis D as a cause of decalcification and tetany When dietary fat was reduced to the point at which the feces were freed from an excess of fatty acids, the faulty absorption of calcium was not materially improved although it seemed to cause some diminution in the negative calcium balance. The loss of lime from the bones was unquestionably a manifestation of D avitaminosis for it could be corrected by ingestion of adequate doses of a vitamin D concentrate (27). Low levels of serum calcium and inorganic phosphorus were likewise restored to normal by this treatment and the symptoms of tetany disappeared.

SUMMARY

1 Case histories and metabolic studies on four patients with idiopathic steatorrhea are presented

2. The subjects exhibited great individual variation in their tolerance of fat but the fecal excretion of fat in each subject was nearly constant for a given régime

3 A very low dietary intake of fat prevented steatorrhea. There was no evidence that steatorrhea was caused by an exceedingly high excretion of fat into the intestine.

4 The tolerance of fat was not improved in one instance by replacing dietary starch with monosaccharide, in another patient a low starch diet seemed to prevent diarrhea and markedly increase the ability to absorb fat.

5 Administration of sodium glycerophosphate and ox bile made the steatorrhea of one patient worse.

6 Ingestion of an alkaline phosphate, a calcium salt, or both caused an increase in the total amount of split fat in the feces

7 Reduction in the intake of calcium facilitated the absorption of fatty acids. This procedure resulted in the loss of calcium and phosphorus from the body in a subject with mild steatorrhea.

8 Increase in dietary fat, when accompanied by steatorrhea, increased the loss of calcium in the feces at the expense of calcium stored in the body

9 Moderately high levels of calcium and phosphorus intake, in the absence of vitamin D, failed to produce consistently positive balances of these elements

10 The ingestion of a phospholipid by one subject was accompanied by a considerable retention of phosphorus, an increase in retention of nitrogen and a loss of calcium. Reasons for the belief that part of the phosphorus was retained as phospholipid have been cited.

11 Rather high values for fecal nitrogen were encountered in one case. These appeared to parallel the fecal lipid and water. In periods free from fatty diarrhea there was no increase in fecal nitrogen.

BIBLIOGRAPHY

- 1 Bennett, T. Izod, Hunter D. and Vaughn, J. M., Idiopathic steatorrhea (Gee's Disease) A nutritional disturbance associated with tetany, osteomalacia and anemia. *Quart. J. Med.*, 1932, n. s. 1, 603
- 2 Castle, W. B., Rhoads, C. P., Lawson, H. A. and Payne, G. C., Etiology and treatment of sprue. *Arch. Int. Med.*, 1935, 56, 627
- 3 Fairley N. H., Tropical sprue with special reference to intestinal absorption. *Tr. Roy. Soc. Trop. Med. and Hyg.*, 1936, 30, 9
- 4 Hansen, K., *Einheimische—europäische—Sprue ihre Symptomatologie und Pathogenese*. Deutsche med. Wochenschr., 1937, 63, 849 and 891
- 5 Manson Bahr Philip and Willoughby Hugh, Studies on sprue with special reference to treatment. *Quart. J. Med.*, 1929-30, 23, 411

- 6 MacLean, A. B and Sullivan, R. C, Carbohydrate tolerance in infants and in young children with celiac disease. *Am. J Dis Child.*, 1929, 38, 16
- 7 Ross, C W, Intestinal absorption in celiac disease with some remarks on effect of liver extract on carbohydrate metabolism *Tr Roy Soc. Trop Med and Hyg*, 1936, 30, 33
- 8 Thaysen, Th. E. Hess, *Non-tropical Sprue*. Oxford University Press, London, 1932
- 9 Parsons, Leonard. See Fairley, N H, Tropical sprue with special reference to intestinal absorption. *Tr Roy Soc. Trop Med and Hyg*, 1936, 30, 9 Discussion by Parsons
- 10 Cohn, Edwin J, Minot, G R, Alles, G A., and Salter, W T, The nature of the material in liver effective in pernicious anemia. II *J Biol Chem*, 1928, 77, 325
- 11 Parsons, Leonard, Celiac disease. *Am J Dis Child.*, 1932, 43, 1293
- 12 Mogensen, Erik. Three cases of idiopathic steatorrhea (Gee-Thaysen's Disease) *Quart. J Med*, 1937, n. s 6, 119
- 13 Fairley, N H, Tropical sprue and its modern treatment. *Brit. M J*, 1934, 2, 1192
- 14 Haas, Sidney V, The value of the banana in the treatment of celiac disease. *Am. J Dis Child.*, 1924, 28, 421
- 5 Morse, J L., Celiac disease. *New England J Med*, 1931, 204, 667
- 16 Miller, Reginald See Fairley, N H., Tropical sprue with special reference to intestinal absorption *Tr Roy Soc. Trop Med. and Hyg*, 1936, 30, 9 Discussion by Miller
- 17 Bauer, Walter and Marble, Alexander, Studies on the mode of action of irradiated ergosterol. II Its effects on the calcium and phosphorus metabolism of individuals with calcium deficiency diseases *J Clin Invest.*, 1932, 11, 21
- 18 Hanes, F M and McBryde, Angus, Identity of sprue, nontropical sprue and celiac disease. *Arch. Int. Med.*, 1936, 58, 1
- 19 Miller, D K. and Barker, W Halsey, Clinical course and treatment of sprue. *Arch Int. Med.*, 1937, 60, 385
- 20 Miller, D K. and Rhoads, C P., The effect of liver extract on the small intestine of patients with sprue. *Am. J M Sc.*, 1936, 191, 453
- 21 Bassett, S H., Mineral exchanges of man. V Balances of electrolytes in a case of hyperparathyroidism *J Nutrition*, 1935, 9, 323
- 22 Chesney, Jack and McCoord, A. B, Vitamin A of serum following administration of haliver oil in normal children and in chronic steatorrhea. *Proc. Soc. Exper Biol. and Med.*, 1934, 31, 887
- 23 Fowweather, F S, The determination of the amount and the composition of the fat in the feces I Investigation of a "wet method" and comparison with the "dry method" *Brit. J Exper Path*, 1926, 7, 15
- 24 Kumagawa, N and Suto, K, Ein neues Verfahren zur quantitativen Bestimmung des Fettes und der unverseifbaren Substanzen in tierischen Material nebst der Kritik einiger gebräuchlichen Methoden. *Biochem Ztschr.*, 1908, 8, 212
- 25 Fanconi, G, Der intestinale Infantilismus und ähnliche Formen der chronischen Verdauungsstörung Ihre Behandlung mit Früchten und Gemüse. *Abhandlungen a. d. Kinderhk.*, 1928, 21, 1
- 26 Badenoch, Eleanor and Morris, Noah, Studies in celiac disease. I. Carbohydrate metabolism *Quart J Med*, 1936, n. s 5, 227
- 27 Bassett, S H, Keutmann, E H, Hyde, H v Z and Van Alstine, H E, Metabolism in idiopathic steatorrhea II Effect of liver extract and vitamin D on calcium, phosphorus, nitrogen, and lipid balances *J Clin. Invest.*, 1939, 18, 000
- 28 Verzar, F and Laszt, L, Untersuchungen über die Resorption von Fettsäuren. *Biochem Ztschr.*, 1934, 270, 24
- 29 Macrae, Olive and Morris, Noah, Metabolism studies in celiac disease. *Arch Dis Child.*, 1931, 6, 75
- 30 Gamble, J L, Ross, G S and Tisdall, F F, Studies of tetany I The effect of calcium chloride ingestion on the acid-base metabolism of infants *Am J Dis Childhood*, 1923, 25, 455
- 31 Herter, C A, *On Infantilism from Chronic Intestinal Infection* The Macmillan Company, New York and London, 1908
- 32 Lehdorff, Heinrich and Mautner, Hans, Die Coeliakie. *Ergebn. d. inn. Med. u. Kinderh.*, 1932, 42, 213
- 33 McCrudden, F H and Fales, H L, Complete balance studies of nitrogen, sulphur, phosphorus, calcium and magnesium in intestinal infantilism. *J Exper Med*, 1912, 15, 450
The nature and origin of the nitrogenous compounds in the feces in infantilism. *J Exper Med*, 1913, 17, 20
- 34 Weir, J F and Adams, Mildred, Idiopathic steatorrhea Metabolic study of a patient with reference to utilization of nitrogen and fat. *Arch. Int. Med*, 1935, 56, 1109
- 35 Linder, G C and Harris, C F, Calcium and phosphorus metabolism in chronic diarrhea with tetany *Quart. J Med.*, 1929-30, 23, 195
- 36 Brüll, Lucien, *Maladie coeliaque chez l'adulte, avec contributions à l'étude du métabolisme calcique*. *Bull. Acad. roy de méd. de Belgique*, 1934 14, 25

METABOLISM IN IDIOPATHIC STEATORRHEA. II EFFECT OF LIVER EXTRACT AND VITAMIN D ON CALCIUM, PHOSPHORUS, NITROGEN, AND LIPID BALANCES

By SAMUEL H. BASSETT E. HENRY KEUTMANN, HENRY VAN ZILE HYDE, HELEN E. VAN ALSTINE

(From the Department of Medicine School of Medicine and Dentistry University of Rochester and the Medical Clinic of the Strong Memorial and Rochester Municipal Hospitals Rochester New York)

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Amelioration of the symptoms of tropical sprue, particularly the anemia and gastro-intestinal disturbances, has followed the oral and parenteral administration of liver or liver extracts (1, 2, 3, 4). It has been claimed that similar treatment is effective in the treatment of idiopathic steatorrhea (non tropical sprue) (5). Barker and Rhoads (6), in a study of the blood lipids in sprue, came to the conclusion that liver extract must exert some specific effect on intestinal absorption. The plasma lipids of the treated cases increased after a meal containing fat, while those patients, who received only a sprue diet, failed to show a similar increase during the test. Ross (7) on the other hand in an investigation of celiac disease (a condition perhaps identical with idiopathic steatorrhea) was unable to demonstrate any effect on the absorption of carbohydrate after injections of liver extract (campolon). He believed that this form of treatment improved the utilization of intravenous glucose.

Whether identical metabolic defects exist in these three diseases is still an open question. It, therefore, seems pertinent to describe the results of balance studies of patients undergoing treatment with liver extract, who have never resided in the tropics and yet have presented the syndrome of sprue. The term idiopathic steatorrhea as used in this connection has been considered synonymous with non tropical sprue.

This report supplements a previous paper (8) and deals specifically with (a) the effect of parenterally administered liver extract on lipid and mineral balances, (b) the effect of diet and other therapeutic procedures on the level of calcium and inorganic phosphorus in the serum, and (c) the effect of administration of vitamin D concentrates

PRESENTATION OF DATA

The four patients whose case histories have been given in detail elsewhere (8) all received parenteral liver extract and a vitamin D concentrate during some period of the investigation. The diets and methods of investigation were the same as those used previously (8).

Liver extract. Three of the patients, J B, R G, and P A., were treated while resident in the metabolic unit. The fourth patient, S B, received liver therapy while on the general ward and since dietary control was inadequate balances could not be kept. The impressions gained in his case have been summarized in his case report (8). Lilly's liver extract (concentrate, N.N.R.)¹ was given to the other patients by intramuscular injection.

Case J B received 5 ml. daily for 6 days (Periods 35 and 36, Table I). At this time she was receiving Diet V believed to contain 115 grams of fat, the amount actually found at a later analysis was 67 grams. Before this fact was established, the decrease in fecal lipid and the more normal appearance of the stools were considered an effect of the liver extract. Although unable to pursue the investigation further in the metabolism unit on the return of the patient to the general ward she was induced to take Diet II for a week. Injections of liver were continued and feces were collected on the last 4 days of this period. They were soft and gray. Analysis for fatty acids showed no noticeable change from that of the early control periods while in the metabolic unit.

¹ The liver extract was contributed by Eli Lilly and Company through the courtesy of Mr George B. Walden.

TABLE I
Lipid, nitrogen, calcium, and phosphorus metabolism during administration of liver extract

Periods	Diet number	Number of days	Total liver extract	Daily lipid intake	Daily feces						Daily balances			Weight
					Weight	Fatty acids	Total lipid	Ca	P	N	Calcium	Phosphorus	Nitrogen	
			ml	grams	grams	grams	per cent of dry weight	grams	grams	grams	grams	grams	grams	kgm
CASE J B														
Control 7-11	II	15	None	100	197	15.0	37.5	1.33	0.73	1.59	-0.08	+0.06	-0.14	47.25
Liver 35*	V*	3	15	67	167	11.3	34.7	1.37	0.58	1.85	-0.44	-0.12	+0.74	47.73
Liver 36*	V*	3	15	67	85	6.4	30.2	0.86	0.38	1.11	+0.08	+0.01	+0.91	48.69
Liver 37†	II†	4	16	100		14.3	40.0							
CASE R. G														
Control 1-4	VI	13	None	105	415	44.0	58.0	1.28	0.96	3.15	-0.197	-0.036	+0.59	53.10
Liver 5-7	VI	9	45	105	511	58.0	52.0	1.67	1.30	3.82	-0.587	-0.365	-0.08	53.23
Liver 8-12	VI	15	70	105	432	50.0	57.6	1.50	1.10	3.60	-0.417	-0.210	+0.09	53.17
CASE P A.														
Control 1-3	VI	12	None	105	185	11.5	32.5	0.96	0.53	2.62	+0.103	-0.002	+0.36	44.82
Liver 4-8	VI	27	115	105	174	11.8	36.0	0.98	0.51	2.35	+0.074	+0.017	+0.81	46.08
Liver 9-10	VI	12	55	105	166	11.8	36.0	0.92	0.41	2.31	+0.153	+0.093	+1.48	46.56
Liver 14-16	VII	12	30											
Liver 17-19	VI	15	30	105	178	12.3	34.3	1.04	0.61	2.56	+0.019	+0.058	+0.86	47.48

* Control on Diet V not obtained See text for interpretation of results

† Period 37 carried out on general medical division

It seems unlikely that the liver extract was responsible for the improvement in steatorrhea, rather the *decrease in fecal lipid was the result of a different diet and a lower intake of fat*. The results demonstrate the advisability of actually analyzing the diet for fat rather than depending upon an estimation of the amount of fat based upon published tables.

Both Cases R. G and P A were given Diet VI and after suitable control periods the daily administration of liver extract was begun without change of diet. R. G received 27 consecutive intramuscular injections of liver extract of 5 cc. each. Data typical of this experiment have been summarized in Table I. No effects were noted which could be attributed to the medication. There was no increase in reticulocytes, and the number of red blood cells and the concentration of hemoglobin remained unaffected. After the close of Period 13 no metabolic observations were made for a week owing to a mild respiratory infection. Beginning with Period 14 the diet was changed to one low in calcium (Diet VII) but

the intake of fat was maintained at the previous level. Injections of liver were continued for eleven days more. There was still no effect attributable to the medication.

Case P A was given 34 intramuscular injections of liver extract of 5 ml each. An occasional day was missed but the injections were in the main consecutive. His anemia remained unchanged. Fecal weight decreased moderately but the amount of fatty acid excreted daily did not differ from the control periods. While receiving an adequate intake of calcium and phosphorus, balances of these elements were for the most part consistently positive, as were nitrogen balances. Analysis of the results obtained in individual periods revealed no evidence that the slightly greater retentions of calcium, phosphorus, and nitrogen in Periods 9 and 10 (Table I) were more than a matter of chance. It is highly improbable that they were in any way connected with the administration of the liver.

Effect of diet during vitamin D deficiency on the concentrations of calcium and phosphorus in

the serum Case J B The inverse relation between Ca and inorganic P in the serum presumably accounted for the lower calcium while ingesting Diets I and II and the higher calcium when on Diet III (9) (See Figure 1) Analysis of the dietary factors associated with these changes brings out the following points. The Ca P ratio of the high fat diet was 0.8 and of the low 1.23. These two diets caused a marked difference in the paths of phosphorus excretion. On the high fat diet with the low Ca P ratio (Diets I and II) the excretion of urinary phosphorus was about twice as great as on the low fat diet. The diversion of phosphorus from the bowel on this diet is explicable on two grounds, (a) the low Ca P ratio left an excess of phosphorus uncombined with alkaline earths in the intestine which was then absorbed and excreted in the urine, (b) the combination of calcium with fatty acids to form soaps (10) decreased the amount of phosphorus bound to alkaline earths still further and left more phosphorus available

for absorption and excretion in the urine (Table II)

The rise in concentration of inorganic phosphorus in the serum seems to have been the result of this greater absorption of phosphorus, and the ultimate effect the same as the administration of an inorganic phosphate by mouth during a later period (Period 22, Figure 1), when tetany was produced. The ease with which tetany may be induced by increasing the intake of phosphorus in vitamin D deficient children and rats has been discussed by Karelitz and Shohl (11). The adult patient with steatorrhea and D avitaminosis is no exception to this rule, for the administration of an inorganic phosphate or a diet with low Ca P ratio and high content of fat is capable, in some instances at least, of depressing the concentration of calcium in the serum to dangerously low levels.

Although there was definite evidence of loss of calcium and phosphorus from the body when the low fat diet with a high Ca P ratio (Diet

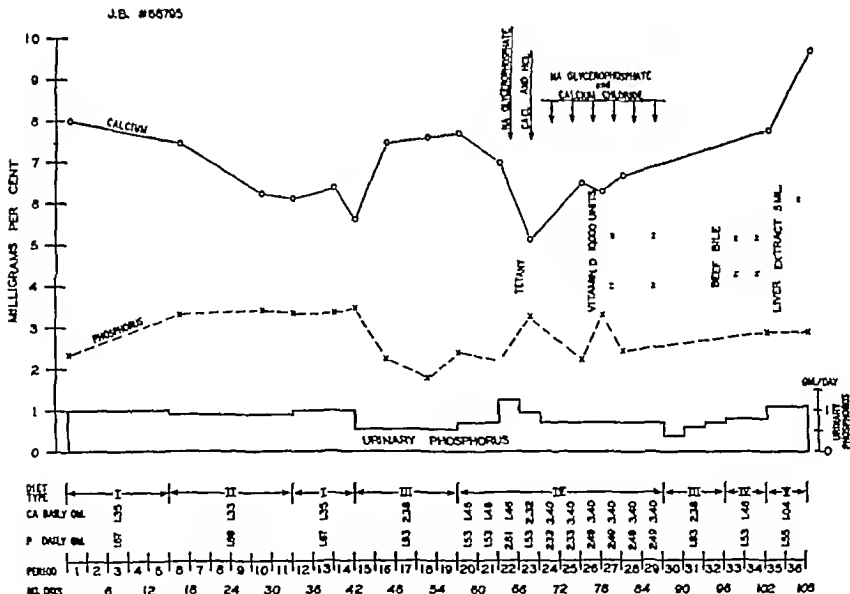


FIG. 1. EFFECT OF DIET AND MEDICATION ON THE SERUM CALCIUM AND INORGANIC PHOSPHORUS OF PATIENT J. B.

TABLE II
Fat, calcium, and phosphorus metabolism in Case J B

Periods	Number of days	Diet			Daily medication	Daily calcium balance			Daily phosphorus balance		
		Number	Fatty acid	Ca P ratio		Urine	Stool	Balance	Urine	Stool	Balance
			grams per day			grams	grams	grams	grams	grams	grams
1-5	15	I	104	0.8	Vitamin D 1 m 10,000 units	0.07	1.26	+0.02	1.07	0.45	+0.15
7-11	15	II	100	0.8		0.08	1.33	-0.08	0.90	0.73	+0.06
12-13	6	I	104	0.8		0.08	1.23	+0.04	1.06	0.57	+0.04
16-19	12	III	2.8	1.23		0.11	2.47	-0.20	0.59	1.36	-0.02
22	3	IV	113.4	0.52*		0.04	2.32	-0.90	1.29	1.67	-0.15
23	3	IV	113.4	1.52*		0.05	2.13	+0.14	0.93	1.11	-0.51
26-29	12	IV	113.4	1.37*		0.06	3.46	-0.12	0.78	1.71	0.00
30	3	III	2.8	1.23		0.06	2.91	-0.59	0.40	1.57	-0.04
31	3	III	2.8	1.23		0.07	2.12	+0.19	0.63	1.25	+0.05
32	3	III	2.8	1.23		0.10	2.39	-0.11	0.46	1.35	-0.12
33	3	IV	113.4	0.95		0.06	2.45	-1.05	0.78	1.30	-0.55
34	3	IV	113.4	0.95		0.07	1.27	+0.12	0.79	0.68	+0.06
Observations discontinued for five days											
35	3	V	67	0.67	Liver extract 5 ml	0.11	1.37	-0.44	1.09	0.58	-0.12
36	3	V	67	0.67	Liver extract 5 ml	0.10	0.86	+0.08	1.16	0.38	+0.01

* Includes Ca given as CaCl_2 and P given as sodium glycerophosphate

I) was given to J B, serum calcium increased and serum inorganic phosphorus decreased (Figure 1 and Table II, Periods 15 to 19). The serum proteins varied between 6.0 and 6.5 grams per cent in Periods 12 to 19 and do not appear to have been a factor in increasing the calcium concentration. From the work of Liu *et al* (12) a high Ca P ratio in a diet may be expected to decrease the concentration of inorganic P in the serum and to decrease its excretion in the urine. When the dietary Ca P ratio was above 1 and the amount of fatty acids in the feces negligible (Periods 15 to 19), a large part of the phosphorus entering the intestine was fixed there as an insoluble phosphate of calcium. The absorption of phosphorus was depressed and its concentration in the serum lowered.

Case R G While the explanation given above seemed valid for J B, interpretation of the data in Case R G (Figure 2) proved to be much more difficult. The levels of calcium and inorganic phosphorus in the serum were determined on numerous occasions, but were omitted in Periods 14 to 17 when the intakes of calcium and phosphorus were lowest. It is, therefore, possible that Figure 2 does not present an entirely unbiased picture of the changes in the blood. These periods would have been of considerable

interest since most of the dietary phosphorus appears to have been excreted in the feces (Table III).

In spite of the deficiencies in analysis of the blood, there were enough data to demonstrate a considerable degree of constancy in the level of serum inorganic phosphorus prior to treatment with vitamin D. In view of the findings in the previous case this was quite unexpected, for the intake, absorption, and urinary excretion of phosphorus varied considerably. The serum calcium on the other hand fluctuated in much the same manner as in Patient J B. The highest level of calcium was observed at the close of Period 19 when calcium equilibrium had been established for a few days, and was apparently the result of the effect of the low fat diet, the lowest serum calcium occurred after two days of diarrhea brought on by ingestion of the high fat diet which was given in Period 30. The balances of lime and phosphorus at this time were not excessively negative when compared with previous periods on the same diet, and the low level of calcium in the blood can hardly be accounted for on the basis of rapid excretion of calcium into the bowel.

When one considers serum calcium and inorganic phosphorus together, it is clear that some factor, other than an inverse relation between the

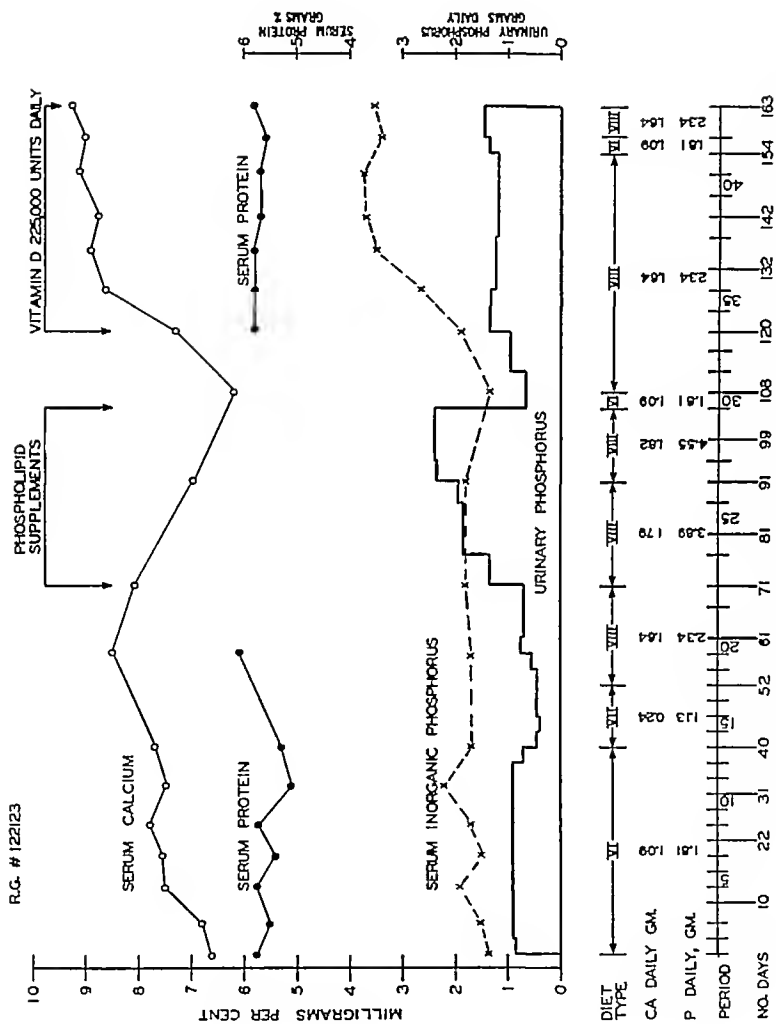


FIG. 2. EFFECT OF DIETARY CHANGES AND VITAMIN D ON THE SERUM CALCIUM AND INORGANIC PHOSPHORUS OF PATIENT R. G.

Liver extract was administered intramuscularly in periods 5 to 18.

TABLE III
Case R G Fat, calcium, and phosphorus metabolism
 (All values are daily averages for the respective periods)

Period		Diet					Feces		Excretion				Balances			Serum*		
Number	Days	Number	Supplement or medication	Fatty acid	Ca	P	Weight	Fatty acid	Calcium		Phosphorus		Ca	P	N	Ca	P	Total protein
				grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm per cent	mgm per cent	grams per cent
1	3	VI		104.0	1.092	1.808	359	86.2	0.005	1.208	0.840	0.968	-0.221	0.000	+1.30	6.67 ⁽¹⁾	1.36	5.74
2	3	VI		104.0	1.092	1.808	198	22.6	0.003	0.670	0.896	0.508	+0.419	+0.404	+1.91			
3	4	VI		104.0	1.092	1.808	503	64.8	0.003	1.830	0.895	1.222	-0.743	-0.409	-0.81	6.82 ⁽¹⁾	1.54	5.53
4	3	VI		104.0	1.092	1.808	392	45.7	0.006	1.146	0.903	0.918	-0.060	-0.011	+0.43			
5	3	VI	Liver extract 5 ml.	104.0	1.092	1.808	997	67.3	0.005	2.173	0.860	1.836	-1.086	-0.888	-1.53	7.54 ⁽¹⁾	1.92	5.74
6	3	VI	Liver extract 5 ml.	104.0	1.092	1.808	824	44.3	0.005	1.095	0.836	0.785	-0.008	+0.187	+1.68	7.63 ⁽¹⁾	1.51	5.41
7-8	6	VI	Liver extract 5 ml.	104.0	1.092	1.808	516	63.3	0.007	1.782	0.927	1.285	-0.697	-0.404	-0.28			
9	3	VI	Liver extract 5 ml.	104.0	1.092	1.808	242	39.1	0.007	1.018	0.956	0.720	+0.072	+0.102	-0.66	7.8 ⁽¹⁾	1.69	5.71
10	3	VI	Liver extract 5 ml.	104.0	1.092	1.808	495	56.3	0.009	1.833	0.916	1.243	-0.570	-0.351	+1.36			
11	3	VI	Liver extract 5 ml.	104.0	1.092	1.808	541	69.4	0.008	1.876	0.866	1.300	-0.592	-0.358	-0.05	7.48 ⁽¹⁾	2.2	5.11
12	3	VI	Liver extract 5 ml.	104.0	1.092	1.808	378	42.2	0.009	1.353	0.866	0.956	-0.270	-0.034	+0.66			
13	3	VI	Liver extract 5 ml.	104.0	1.092	1.808	744	68.1	0.006	1.813	0.790	1.512	-0.727	-0.495	-0.03	7.68 ⁽¹⁾	1.73	5.30
14-17	12	VII	Liver extract 5 ml.	105.0	0.229	1.017	519	32.7	0.005	0.624	0.455	0.879	-0.400	-0.317	-0.43			
18	3	VIII		15.5	1.090	1.561	310	10.9	0.005	0.980	0.463	0.900	+0.105	+0.198	+0.70			
19	3	VIII		15.5	1.090	1.561												
20	3	+ 50% VIII		23.2	1.635	2.342	140	9.5	0.010	1.460	0.655	1.226	-0.108	+0.070	+1.48	8.53 ⁽¹⁾	1.72	6.17
21-22	10	+ 50% VIII		23.2	1.635	2.342	155	6.7	0.016	1.942	0.698	1.537	-0.323	+0.105	+1.63			
23	6	+ 50% VIII	Phospholipid 50 grams	67.4	1.790	3.890	163		0.009	1.850	1.351	1.730	-0.069	+0.809	+3.03	8.1 ⁽¹⁾	1.76	
24-26	14	+ 50% VIII	Phospholipid 50 grams	67.4	1.790	3.890	209		0.012	1.973	1.861	1.844	-0.198	+0.183	+2.40			
27	4	+ 50% VIII	Phospholipid 70 grams	67.6	1.820	4.545	220		0.012	1.992	2.355	2.047	-0.184	+0.143	+2.28	7.02	1.78	
28-29	10	+ 50% VIII	Phospholipid 70 grams	67.6	1.820	4.545	193		0.010	1.837	2.417	1.811	-0.026	+0.317	+2.42			
30	3	+ 50% VIII	None	104.0	1.092	1.808	1067	55.6	0.005	1.166	0.703	1.470	-0.079	-0.365	+0.68	6.18 ⁽¹⁾	1.35	
31-33	12	+ 50% VIII	None	23.2	1.635	2.342	232	7.6	0.008	1.558	0.863	1.240	+0.060	+0.239	+1.51			
34	4	+ 50% VIII	Vitamin D 225,000 I.U.	23.2	1.635	2.342	160	4.5	0.019	1.797	1.335	1.195	-0.181	-0.188	+1.45	7.3 ⁽¹⁾	1.79	5.80
35	4	+ 50% VIII	Vitamin D 225,000 I.U.	23.2	1.635	2.342	135	6.0	0.034	1.170	1.227	0.472	+0.481	+0.548	+1.72			
36	4	+ 50% VIII	Vitamin D 225,000 I.U.	23.2	1.635	2.342	183	7.6	0.015	0.867	1.322	0.499	+0.753	+0.521	+0.18	8.59 ⁽¹⁾	2.66	5.77
37	6	+ 50% VIII	Vitamin D 225,000 I.U.	23.2	1.635	2.342	80	4.5	0.016	0.370	1.026	0.249	+1.249	+1.067	+1.72	8.94 ⁽¹⁾	3.49	5.80
38	4	+ 50% VIII	Vitamin D + butter 50 grams	68.2	1.635	2.342	138	11.2	0.009	0.617	1.187	0.232	+1.009	+0.823	+1.74			
39	4	+ 50% VIII	Vitamin D + butter 50 grams	68.2	1.635	2.342	97	6.9	0.015	0.450	1.245	0.211	+1.170	+0.886	+2.05	8.70 ⁽¹⁾	3.70	5.70
40	4	+ 50% VIII	Vitamin D + butter 50 grams	68.2	1.635	2.342	123	7.7	0.022	0.520	1.190	0.325	+1.093	+0.827	+1.34			
41	4	+ 50% VIII	Vitamin D + butter 75 grams	90.7	1.635	2.342	57	6.6	0.022	0.400	1.192	0.204	+1.213	+0.946	+3.05	9.17 ⁽¹⁾	3.76	5.70
42	3	VI	Vitamin D 225,000 I.U.	104.0	1.092	1.808	390	24.2	0.005	0.846	1.340	0.410	+0.241	+0.058	-0.24	9.07 ⁽¹⁾	3.46	
43	6	+ 50% VIII	Vitamin D 225,000 I.U.	23.2	1.635	2.342	126	10.1	0.013	0.641	1.435	0.300	+0.981	+0.607	+1.26	9.24 ⁽¹⁾	3.56	5.80

* Numbers in parenthesis refer to the day of period on which blood was taken

two, must have affected the level of calcium (2) better absorption of vitamin D from the diet, Factors which might tend to elevate calcium were (3) a higher concentration of protein in the (1) a state of equilibrium with the diet as ap- serum, and (4) greater activity of the parathy- posed to a previously negative calcium balance, roid glands With the exception of the calcium

balance there was no evidence of the possible effect of any of these factors, and even the evidence derived from a survey of the balances proves to be rather contradictory (Periods 18 to 30)

In attempting to explain the whole situation one might hypothesize a more active participation of the parathyroids in the mechanism for regulating the serum calcium and phosphorus of the second patient (R. G.) The rapid and severe drain upon his reserves of calcium which resulted from steatorrhea and D avitaminosis would, according to the suggestion of Albright and Sulzowitch (13), lower the calcium of the serum and stimulate the parathyroid apparatus. This in turn would accelerate the decalcification of bone, tend to raise the calcium of the serum and depress the inorganic phosphorus by hastening its excretion in the urine. If the rate of excretion of phosphorus by the kidney were sufficiently rapid, no appreciable rise of inorganic phosphorus in the blood would occur unless the amount passing into the blood from the intestine were very large. Telfer has suggested that there is a primary defect in the absorption of phosphorus in celiac disease (14). The data we obtained while observing the effect of vitamin D lend some support to this concept. Nevertheless, even in the most severe cases of the disease, considerable quantities of phosphorus were absorbed and excreted in the urine, and it is possible that the limiting factors were diarrhea and the presence of large quantities of calcium in the bowel with which phosphorus may have combined to form insoluble phosphates.

Vitamin D Skeletal decalcification, excessive loss of calcium in the feces, low serum calcium and inorganic phosphorus, and failure to absorb calcium when there was no steatorrhea all pointed to a deficiency of vitamin D in Subjects J B, R. G. and S B.

When studying the first patient, J B, it was feared that even a vitamin D concentrate might escape absorption if given by mouth while the intake of fat was high. To avoid this possibility one gram of a solution of viosterol in oil (Squibb) was given daily by intramuscular injection for 12 days (Periods 26 to 29, Table II). This was equivalent to 120 000 international units of vitamin D, enough according to Hannon

et al (15) to establish a prolonged remission in osteomalacia. No immediate change in the concentration of serum calcium occurred, nor were the calcium and phosphorus balances or their paths of excretion affected. Believing that the excessive excretion of fatty acid might be interfering with calcium absorption the patient was returned to the low fat diet (III) for three periods of 3 days each (Table II Periods 30 to 32). As before, there was a very prompt and marked reduction in fecal lipid and reduction in the amount of fecal water, but the time allotted was too short to study adequately the effect on calcium and phosphorus balance. A moderate retention of calcium was observed in Period 31 but Period 32 was spoiled by administration of bile. Twenty days after the last dose of viosterol, the serum calcium had increased from 6.3 to 7.7 mgm. per cent and six days later to 9.7 (Figure 1). The interpretation of these changes is uncertain. They may have been caused by a delayed effect of viosterol caused by slow absorption of the oily solution. Another possibility for the delayed action of vitamin D may have been diarrhea produced by administration of bile and lasting until the end of Period 34. Unfortunately, 5 days intervened at the end of this period when no balance studies could be done. Whether calcium and phosphorus retention occurred in this interval is not known. A further complication was introduced by administration of liver extract in Periods 35 to 36. However, since liver extract did not seem to influence the levels of serum calcium and inorganic phosphorus of the other subjects who received it, we are inclined to minimize its importance.

The inconclusive effects of this experiment are to be contrasted with those obtained on Subjects R G P A., and S B., who were given the vitamin orally and in much higher dosage.* Subjects R G. and S B. received the vitamin D concentrate while ingesting Diet VIII. Both subjects had had tetany, and the level of calcium and inorganic phosphorus in the serum was very low when treatment was started.

*The vitamin D concentrate was contributed by the Winthrop Chemical Company Inc. through the courtesy of Mr F. E. Houghton. It was described as a solution of crystalline vitamin D in oil having a potency of 1 000 000 USP vitamin D units per gram.

The effect on R G was prompt. Almost immediately there was an increase in the phosphorus content of the urine (Figure 3) followed in a few days by a marked decrease in fecal phosphorus (Table III). The latter more than offset increased urinary excretion and the balance became strongly positive. Fecal calcium also decreased markedly without any appreciable increase in urinary calcium. The net effect was a considerable retention of both elements. The changes in the serum were definite, both calcium and inorganic phosphorus rising toward normal (Figure 2).

The fecal lipids were low on this diet and remained unaffected by the vitamin. After 18 days (Periods 34 to 37) butter supplements were added to the diet without producing appreciable change in the composition of the feces. In Period 41 the intake of fatty acid had been increased in this manner to 91 grams daily and now approached the amount given in the control diet. The latter (Diet VI) was substituted in Period 42, and symptoms of steatorrhea developed within 24 hours. As Period 30 was comparable to 42 in all respects except for the administration of the vitamin, it served as a useful standard of reference. There was appreciably less steatorrhea in Period 42, and enough calcium and phosphorus were absorbed to produce positive balances. The suggestion is, therefore, rather strong that steatorrhea had been lessened by relief of the vitamin deficiency. The mechanism of the effect remained obscure. It may have been related to better absorption of calcium from the intestine, to decreased intestinal irritability accompanying a higher level of calcium in the blood and tissues, or to some factors at present unknown. Johnson (16) thought that, when viosterol was administered to a patient with an ileal fistula, the rate of propulsion of the contents of the small bowel decreased giving a longer absorptive period.

Case S B's metabolism was followed for a much shorter time, but the data given in Table IV show a similar response to vitamin D therapy.

Case P A's steatorrhea was so mild that there was very little tendency to diarrhea, even while ingesting the diet (VI) to which R G gave evidence of marked intolerance. He received the vitamin D concentrate together with the high fat ration in Periods 20 to 27 (Table V). There

were no definite signs of D avitaminosis prior to treatment. Serum calcium and inorganic phosphorus were normal and did not increase with treatment. The excretion of urinary phosphorus increased as it had in the other patients (Figure 3), but the main effect was on the feces. Decreased excretion of fecal calcium and phosphorus led to a good retention of both elements. The excretion of fecal fatty acids remained unchanged.

It is evident that the oral administration of a suitable vitamin D concentrate proved an effective means of correcting the calcium and phosphorus deficiencies of severe and mild steatorrhea. The solubility of the vitamin in fats has been suggested as a cause of its poor absorption (17), and seems adequate reason for its administration in connection with a diet that reduces fecal lipids to a low level. This does not necessarily imply the rigid exclusion of dietary fats in all cases (cf., Case P A).

With the exception of J B, it is probable that the dose of vitamin D was considerably greater than necessary. Obviously this point requires further study. Improvement in general health and absence of untoward symptoms seemed to exclude any toxic action.

Once the body has been thoroughly saturated with the vitamin, it is excreted or inactivated quite slowly and continuous administration may not be necessary (15, 18, 19). One of our patients, R G, has maintained the calcium and inorganic phosphorus concentrations of his serum at normal levels without additional medication for more than six months. It has perhaps been possible for him to absorb sufficient vitamin D from his diet for maintenance, especially since he has followed dietary instructions faithfully and has had no diarrhea. Patient S B, on the other hand, has shown a definite tendency to develop hypocalcemia and hypophosphatemia when his food was no longer fortified with viosterol. The time interval involved is not known accurately, but relapse has occurred in less than eleven months (see Case report (8)). Direct comparison of the duration of the vitamin D effect in the two patients is not possible because of the different initial dosage and their different modes of living.

There are some points of interest in regard to

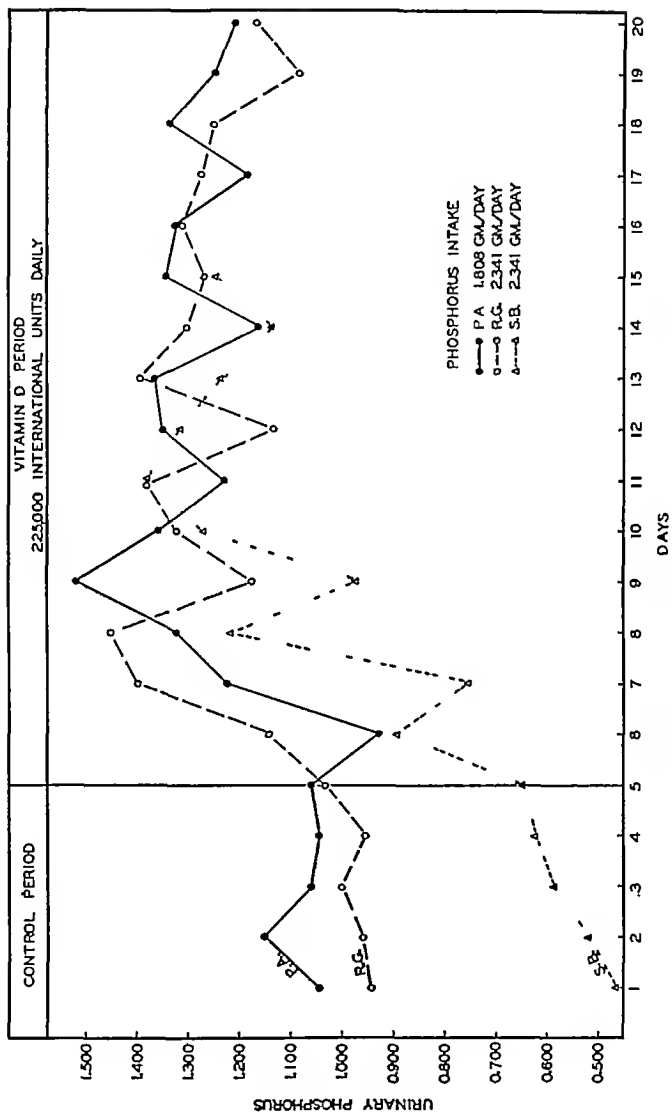


FIG. 3 INCREASE IN THE EXCRETION OF URINARY PHOSPHORUS ACCOMPANYING THE ORAL ADMINISTRATION OF A VITAMIN D CONCENTRATE

TABLE IV

Case S.B. Effect of vitamin D on calcium and phosphorus metabolism

Period	Number of days	Diet		Daily medication	Daily feces				Daily calcium			Daily phosphorus			Serum		
		Number	Fatty acid		Wet weight	Dry weight	Total fatty acid	Nitrogen	Urine	Stool	Balance	Urine	Stool	Balance	Ca	P	Total protein
			grams per day		grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	mgm per cent	mgm per cent	per cent
2	4	VIII*	23.2	None	210	58.3	17.3	3.18	0.016	2.255	-0.640	0.519	1.698	+0.125	6.8	2.4	4.7
4	4	VIII*	23.2	Vitamin D 225,000 I U	173	53.0	18.2	2.28	0.013	1.585	+0.040	0.913	1.028	+0.401	7.2	2.8	4.8
5	4	VIII*	23.2	Vitamin D 225,000 I U	142	46.4	20.1	2.04	0.010	1.390	+0.240	1.335	0.640	+0.367			
6	3	VIII*	23.2	Vitamin D 225,000 I U	152	41.7	14.8	2.02	0.008	1.180	+0.450	1.240	0.565	+0.537	8.0	3.9	5.3

* Diet increased 50 per cent

TABLE V

Case P.A. Effect of vitamin D administration

Period	Number of days	Diet per day							Medication total	Daily excretion and balances									Daily feces		Body weight
		Number	Protein	CHO	Fatty acids	Ca	P	Calories (approx.)		Calcium			Phosphorus			Nitrogen			Moist weight	Total lipid	
										Urine	Feces	Balance	Urine	Feces	Balance	Urine	Feces	Balance			
1-3	12	VI	98	343	104	1.092	1.808	2900	None	grams 0.027	grams 0.662	grams +0.103	grams 1.283	grams 0.527	grams -0.002	grams 13.06	grams 2.62	grams +0.06	grams 185	grams 13.6	kgm. 45.33
17-19	15	VI	98	343	104	1.092	1.808	2900	Liver extract 30 ml.	grams 0.030	grams 1.043	grams +0.019	grams 1.136	grams 0.614	grams +0.058	grams 12.32	grams 2.56	grams +0.86	grams 178	grams 14.7	kgm. 44.82
20	4	VI	98	343	104	1.092	1.808	2900	Vitamin D 800,000 units	grams 0.030	grams 1.030	grams +0.032	grams 1.175	grams 0.585	grams +0.048	grams 12.57	grams 2.60	grams +0.57	grams 163	grams 15.9	kgm. 47.70
21	4	VI	98	343	104	1.092	1.808	2900	Vitamin D 800,000 units	grams 0.039	grams 0.800	grams +0.253	grams 1.330	grams 0.287	grams +0.191	grams 12.50	grams 2.38	grams +0.86	grams 157	grams 15.2	kgm. 47.77
22-25	10	VI	98	343	104	1.092	1.808	2900	Vitamin D 3,225,000 units	grams 0.070	grams 0.700	grams +0.222	grams 1.370	grams 0.260	grams +0.178	grams 11.74	grams 2.26	grams +1.74	grams 137	grams 14.9	kgm. 48.70
26-27	8	VI	98	343	104	1.092	1.808	2900	Vitamin D 800 000 units	grams 0.084	grams 0.685	grams +0.313	grams 1.371	grams 0.242	grams +0.195	grams 11.78	grams 2.07	grams +1.89	grams 128	grams 14.8	kgm. 48.80
28	4	VI	98	343	104	1.092	1.808	2900	None	grams 0.085	grams 0.773	grams +0.254	grams 1.455	grams 0.273	grams +0.080	grams 12.46	grams 2.22	grams +1.06	grams 144	grams 17.3	kgm. 49.34

the action of vitamin D which remain to be considered Albright and Sulkowitch (13) have stated that massive doses of the vitamin increase the excretion of phosphorus in the urine. This effect was particularly noteworthy in Subjects R G and S B. In the latter, the paths of excretion were completely reversed. The phosphorus in the urine during the second period on the vitamin was about 2.5 times as great as during the control period. (Compare Periods 2 and 5, Table IV.) All of this extra phosphorus seems to have been derived from increased absorption from the gut. While the fecal calcium decreased, it was not reduced as much as might have been expected from the decrease in fecal phosphorus. The result of treatment of R. G. was essentially the same. The effect of vitamin

D was clearly apparent in the first period in which it was given (Period 34, Table III.) The excretion of phosphorus in the urine increased about 470 mgm a day, but there was little or no change in the fecal excretion of either calcium or phosphorus. An effect on the feces was noted in Period 36. Fecal phosphorus decreased by an average of 768 mgm per day below the control level established in Periods 31 to 33. Fecal calcium fell 388 mgm below its control level. The actual phosphorus balances of both patients were considerably in excess of the theoretical balances (22). These findings are difficult to reconcile with the view that the increased absorption of phosphorus after vitamin D was entirely secondary to the absorption of calcium. The argument might be raised that the absorption of phos-

phorus was secondary to the combined absorptions of calcium and magnesium. Reference to Table VI in which it has been assumed that each millimol of phosphorus was combined with 2 m.eq. of base does not point to any considerable participation of magnesium in the absorption of phosphorus. About all that can be said on the basis of the data at hand is that the vitamin, (a) increased excretion of phosphorus in the urine before it affected the fecal excretion, (b) markedly increased the absorption of both Ca and P from the gut, and (c) appeared to increase the absorption of magnesium.

TABLE VI

Calcium, magnesium and phosphorus in feces before and during vitamin D administration

Period	Ca m.eq. per day	Mg m.eq. per day	Ca + Mg m.eq. per day	P m.eq. per day	Medication
CASE S.B.					
2	112	35	147	109	None
4	79	29	108	67	Vitamin D 225 000
5	69	29	98	41	Vitamin D 225 000
6	59	26	85	36	Vitamin D 225 000
CASE R.G.					
31-33	79	29	108	80	None
34	90	31	121	77	Vitamin D 225 000
35	59	24	83	30	Vitamin D 225 000
36	43	29	72	32	Vitamin D 225 000
37	18	15	33	16	Vitamin D 225 000
38	31	26	57	21	Vitamin D 225 000
39	22	18	40	14	Vitamin D 225 000

COMMENT

The development of deficiency states in steatorrhea and in sprue leads to a vicious circle. The function of the gastro-intestinal tract suffers first as a result of some unknown primary disorder impairing its absorptive power and then from malnutrition and specific deficiencies which further reduce the tolerance for foods which cannot be properly digested and absorbed. Clinical recovery may result from relief of recognizable deficiencies such as macrocytic anemia and osteomalacia, if combined with appropriate dietary therapy. The latter permits restitution of bodily tissues and functions affected by malnutrition.

Our experience with liver extract in treatment

of two patients, one of whom had hypochromic anemia and mild steatorrhea, the other a very mild macrocytic anemia and severe steatorrhea did not point to a specific action of the extract on the fatty diarrhea. The quantities of extract used were in general comparable to or larger than the amounts found effective in tropical and non tropical sprue by other investigators (1, 2, 3, 5). The essential differences were perhaps that our subjects were nearly free from clinical signs of any deficiency which could unquestionably be relieved by liver and in addition were kept upon a rigorously controlled diet, proven in each instance to be associated with steatorrhea. It was hoped that the deliberate use of such a diet, the effect of which on the subject was carefully measured in advance might enable us to distinguish a specific effect of liver extract on steatorrhea, if such existed. The evidence seems to be against a specific effect either on the intolerance for fats or carbohydrates. The latter has been judged by the failure of glucose tolerance to show material improvement (Case reports (8)).

The statement by Verzar (20) that the underlying biochemical defect in steatorrhea is a failure in phosphorylation of fatty acids and glucose awaits clinical confirmation. Since the hypothetical deficiency is a lack of flavin phosphoric acid, and, since liver extract contains this principle (21) one might expect improvement from adequate dosage of liver. Perhaps the amounts we have used were inadequate or the preparation may have been too highly purified (7).

One or more other factors in the vitamin B complex are represented in liver extract. The evaluation of the deficiencies which their lack produces may be difficult or impossible in the human subject particularly when masked by another disease. For example, should the patient with steatorrhea develop the type of digestive disorder not infrequently observed in the pellagra, then liver extract might prove of considerable benefit, especially since the diarrheal disturbances produced by the two syndromes would probably be additive.

Conjecture as to the probable course of events leading to the D avitaminosis leaves at least two alternatives (a) it may be regarded perhaps as among the secondary manifestations of the disease. Malabsorption of fatty acids as suggested

by Linder and Harris (17) would then be considered primary. The high concentration of intestinal fat provides a medium in which the vitamin is readily soluble and hence its uptake by the intestinal epithelium is impaired. A similar explanation would account for the apparent failure of these patients to absorb a vitamin A concentrate. Diarrhea when present must be included as an additional hindrance to absorption. (b) The delayed absorption of glucose in glucose tolerance tests done in a postabsorptive state cannot be readily laid to the mechanical effects of fat and seems to point to a more general impairment of the absorptive power of the gut. Possibly tests of the ability to absorb other simple substances would show a similar delay. If this were found to be the case, then the various deficiency states that arise might be regarded as part of a general failure of intestinal absorption which is obviously intensified by diarrhea.

SUMMARY

1 Prolonged intramuscular administration of liver extract to patients with idiopathic steatorrhea (non-tropical sprue) failed to cause improvement in the absorption of fatty acids, calcium, phosphorus, or nitrogen.

2 In one patient the inverse relationship between calcium and inorganic phosphorus in the serum was found when both these elements were at subnormal levels, before vitamin D was administered. In another patient under the same conditions the level of phosphorus in the serum did not change markedly when the serum calcium changed. It is suggested that this difference in behavior may be owing to difference in activity of the parathyroid glands or difference in rate of absorption of phosphorus from the intestine.

3 The oral administration of large doses of vitamin D caused the following changes:

(a) Increased excretion of phosphorus in the urine before there was evidence of improved calcium absorption.

(b) Increased absorption of calcium from the intestine.

(c) Increased absorption of magnesium from the intestine.

(d) Increased absorption of phosphorus from the intestine. The magnitude of this increase

was such that it was probably not entirely secondary to improved absorption of calcium and magnesium.

(e) Some improvement of fatty acid absorption in two patients. This was interpreted as caused by improved calcium absorption or decrease in the rate of propulsion through the small intestine, and probably not to improvement of the primary disorder.

(f) Improvement of absorption of water and nitrogen from the feces. These likewise were interpreted as secondary effects.

BIBLIOGRAPHY

- 1 Bloomfield, A. L. and Wyckoff, H. A., Remission in sprue following high liver diet. *California and West. Med.*, 1927, 27, 659.
- 2 Castle, W. B., Rhoads, C. P., Lawson, H. A., and Payne, G. C., Etiology and treatment of sprue. *Arch. Int. Med.*, 1935, 56, 627.
- 3 Miller, D. K. and Barker, W. Halsey, Clinical course and treatment of sprue. *Arch. Int. Med.*, 1937, 60, 385.
- 4 Miller, D. K. and Rhoads, C. P., The effect of liver extract on the small intestine of patients with sprue. *Am. J. M. Sc.*, 1936, 191, 453.
- 5 Hanes, F. M. and McBryde, Angus, Identity of sprue, nontropical sprue and celiac disease. *Arch. Int. Med.*, 1936, 58, 1.
- 6 Barker, W. Halsey and Rhoads, C. P., The effect of liver extract on the absorption of fat in sprue. *Am. J. M. Sc.*, 1937, 194, 804.
- 7 Ross, C. W., Intestinal absorption in celiac disease with some remarks on effect of liver extracts upon carbohydrate metabolism. *Tr. Roy. Soc. Trop. Med. and Hyg.*, 1936, 30, 33.
- 8 Bassett, S. H., Keutmann, E. H., Hyde, H. van Z., Van Alstine, H. E., and Russ, E., Metabolism in idiopathic steatorrhea. I The influence of dietary and other factors on lipid and mineral balance. *J. Clin. Invest.*, 1939, 18, 101.
- 9 Peters, J. P. and Van Slyke, D. D., Quantitative Clinical Chemistry Vol I Interpretations. Williams and Wilkins Co., Baltimore, 1931, p. 811.
- 10 Telfer, S. V., Studies in calcium and phosphorus metabolism. IV The influence of free fatty acids in the intestine on the absorption and excretion of the mineral elements. *Quart. J. Med.*, 1926, 20, 1.
- 11 Karelitz, S. and Shohl, A. T., Rickets in rats. II The effect of phosphate added to the diet of ricketic rats. *J. Biol. Chem.*, 1927, 73, 665.
- 12 Liu, S. H., Hannon, R. R., Chou, K. G., Chu, H. I., and Wang, S. H., Calcium and phosphorus metabolism in osteomalacia. III The effects of varying levels and ratios of intake of calcium to phosphorus on their serum levels, paths of excretion and balances. *Chinese J. Physiol.*, 1935, 9, 101.

- 13 Albright, Fuller and Sulkowitch Hirsh W., The effect of vitamin D on calcium and phosphorus metabolism studies on four patients J Clin. Invest., 1938, 17 305
- 14 Telfer S V., Mineral metabolism in celiac disease. Glasgow M. J., 1928 109 306
- 15 Hannon R R., Liu, S H Chu H. I., Wang, S H., Chen, K. C., and Chou, S K., Calcium and phosphorus metabolism in osteomalacia. I The effect of vitamin D and its apparent duration. Chinese M. J., 1934 48 623
- 16 Johnson, Richard M., The absorption and excretion of calcium and phosphorus in three patients with colostomy and ileostomy J Clin. Invest., 1937 16 223
- 17 Lunder G C. and Harris, C. F., Calcium and phosphorus metabolism in chronic diarrhea with tetany Quart J Med., 1929-30 23, 195
- 18 Heymann Walter Metabolism and mode of action of vitamin D V Intestinal excretion of vitamin D J Biol. Chem. 1937 122, 257
- 19 Windorfer, A., Ueber die Vitamin D Resorption bei Verabreichung hoher Dosen (Vitamin D-stoss) Klin. Wchnschr 1938 17 228.
- 20 Verzář F and Laszt, L., Untersuchungen über die Resorption von Fettsäuren. Biochem. Ztschr., 1934 270 24
Verzář F Resorptionsstörungen durch Erkrankungen der Nebennierenrinde. Schweiz. med. Wchnschr., 1937 67, 823
- 21 Elvehjem, C. A. and Koehn, C. J., Studies on vitamin B₂ (G) The non identity of vitamin B₂ and flavins J Biol. Chem., 1935 108 709
- 22 Aub J C., Bauer W., Heath, C., and Ropes, M., Studies of calcium and phosphorus metabolism III. The effects of the thyroid hormone and thyroid disease. J Clin. Invest., 1929 7, 97

RENAL FUNCTION AS A FACTOR IN THE URINARY EXCRETION OF ASCORBIC ACID

By JULIUS SENDROY, JR.¹ AND BENJAMIN F. MILLER²

(From the Hospital of the Rockefeller Institute for Medical Research, New York City)

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The demonstration by Harris Ray, and Ward (1) of a correlation between the urinary excretion of ascorbic acid and the dietary intake has led to many investigations of ascorbic acid metabolism based on the technique suggested by these authors. Sendroy and Schultz (2) have given especial attention to some of the possible factors affecting tests based on urinary excretion values. A urinary excretion test might also be affected by another factor, namely, that of kidney function.

The difference between the ascorbic acid in the test dose and the amount in the urinary output has been ascribed either to a deficiency, leading to storage, or to an increased destruction of the material in the body. Nevertheless, it might be increased especially in disease, by a lowered excretion rate. In the great majority of nutrition studies there has probably been little error involved in the neglect of this factor, or in the tacit assumption that renal function was unvarying or normal in the cases observed. However, since such tests may and, indeed have been applied under conditions where kidney function was undoubtedly abnormal, it seemed desirable to investigate the effect of this factor and to learn something of the mode of renal excretion of ascorbic acid.

Drigalski (3), who studied a variety of pathological conditions, among which were Addison's disease, acute nephritis, and nephrosis (but one case in each condition) concluded that the excretion of ascorbic acid in the urine was independent of changes in kidney function with respect to glomerular or tubular damage. Siwe (4) studied one case of Addison's disease and found a decreased excretion of ingested ascorbic acid. Shortly thereafter, Wilkinson, Manch, and Ashford (5) studied the ascorbic acid urine excretion of three women with Addison's disease, and observed a poor response to test doses

They concluded that the degree of ascorbic acid subnutrition in their cases paralleled the severity of the disease. However, they did not take into consideration the possibility that their results indicated not a disturbance of ascorbic acid metabolism but a functional renal insufficiency, which is one of the well known characteristics of Addison's disease.

Van Eekelen's (6) observation that a surplus excretion of ascorbic acid occurs in the urine when the blood level of about 1.3 mgm. per 100 cc. is exceeded, seems to have been the first indication that a renal threshold was involved. Lund (7) and Faulkner and Taylor (8) have also concluded that the ascorbic acid concentration in the urine is dependent on the serum concentration. The latter authors found a threshold level of about 1.4 mgm. per 100 cc. of serum. Because of the unknown effect of renal impairment on urinary excretion, Wright, Lilienfeld, and MacLenathen (9) have advocated, as a criterion of nutritional saturation of ascorbic acid, a study of the blood curve in addition to measurement of the urinary excretion, after intravenous injection of ascorbic acid. More recently, Wright and MacLenathen (10) have studied a patient who had malignant hypertension, with a nitrogen retention definitely indicative of a diminished renal function. This individual, despite a history of adequate ascorbic acid intake, showed a distinct retention of the injected test dose. The low output in the urine, usually a sign of subnutrition, could therefore be attributed to kidney damage.

On the basis of simultaneous ascorbic acid and inulin clearances in man (in presumably normal individuals) over a wide range of plasma ascorbic acid concentrations Friedman, McGoey, and Ralli (11), and Ralli, Friedman, and Rubin (12) have found further evidence of the threshold nature of ascorbic acid renal excretion.

The present paper is concerned with a comparative study of the effect of

¹ Present address—Department of Medicine, Loyola University School of Medicine, Mercy Hospital, Chicago.

² Present address—University of Chicago, Chicago.

renal impairment on the urinary excretion of ascorbic acid in man. The excretion after the administration of large test doses was determined under conditions as normal as possible with respect to the nutritional and metabolic factors affecting the test. The urinary output was then directly correlated with renal function, which was subsequently determined by simultaneous ascorbic acid and urea clearance tests.

METHOD

As subjects, there were used eight patients previously hospitalized for some form of renal disease, and four normal individuals serving as controls.

Ascorbic acid utilization test

This was not done at this time on the normal subjects, two of whom (Subjects 9 and 10, Table II) had been tested previously. The utilization index for normals has been found by Sendroy and Schultz (2) to vary only within the average limits of 675 ± 55 . The index was determined, however, for each patient, it being ascertained that the diet had been adequate with respect to ascorbic acid, prior to the excretion test. Of these cases, none was disturbed by digestive (anorexia, etc.) or metabolic (fever, etc.) disorders during the test.

The urinary excretion test was carried out, with a few modifications, according to the technique of Sendroy and Schultz (2). The diet during the test contained the minimum of ascorbic acid (less than 12 mgm. daily). After 48 hours, this was supplemented, for the next seven days, by the addition of 250 mgm. of "Redoxon" (l-ascorbic acid, Hoffman-La Roche) daily, taken after breakfast. Fluid intake was somewhat restricted.

Collection of urine specimens At the beginning of a test period, the urine voided at 8 a.m. was discarded. The last specimen for every 24-hour period was obtained at 8 a.m. Although Sendroy and Schultz's method (2) of collection has been shown to give satisfactory results, the following procedure, involving the use of a preservative to prevent ascorbic acid oxidation, was adopted because of its greater convenience and accuracy.

All of the urine voided was quickly transferred to, and stored in, 750 cc. specimen bottles. Usually, two bottles sufficed for the 24-hour collection. Each bottle was prepared for use by the prior addition of a preservative mixture consisting of 75 cc. of 5 N H_2SO_4 solution (approximately 135 cc. of concentrated H_2SO_4 per liter), 0.75 cc. of 0.1 M 8-hydroxyquinoline solution (1.45 grams per 100 cc. 95 per cent ethyl alcohol),* and 1.5 cc. of toluene,

* Sulfuric acid and 8-hydroxyquinoline were suggested for use in urine analyses by Dr. E. S. Guzman Barron (see "Experimental" section). We have found alcohol a better solvent for 8-hydroxyquinoline than dilute sulfuric acid, in that solutions could be kept for use for at least one week without visible color change. Toluene was added to minimize bacterial action.

c.p. Between voidings, and between the time of the last specimen collection and that of the analysis, the bottles were kept stoppered, in a refrigerator, at about 5° C.

Titration of urine specimens For analysis, the total 24-hour output (together with the added preservative) was mixed and measured in a graduated cylinder, from which duplicate samples of from 2 to 20 cc. were withdrawn, depending on the concentration of ascorbic acid present. Water, to bring the total volume to about 48 cc., and 2.5 cc. of glacial acetic acid⁴ were added. Titration with 2,6-dichlorophenol indophenol was carried out as described by Sendroy and Schultz (2), except that the indicator was standardized against a solution of ascorbic acid ("Redoxon") in 2 per cent metaphosphoric acid instead of in water.

Urea and ascorbic acid clearance tests

Simultaneous clearances were done on all subjects, patients and normals, after the completion of the urinary excretion studies. When the clearance test was begun some time after the utilization test was finished, large doses of ascorbic acid were fed during the interval, in order to avoid the complications of a possible "undersaturation."

The clearances were determined by the technique used by Van Slyke and coworkers, with modifications as described for urea by Van Slyke, Page, Hiller, and Kirk (13). On the day of the experiment, the subject was deprived of breakfast. At 6 a.m. he received 200 cc. of water *per os*, followed at 7 a.m. by 500 mgm. of "Redoxon" and 200 cc. of water. At 8 a.m. an additional 200 cc. of water were given. At 9 a.m. the first of two one-hour periods was started. At the middle of each period of urine collection, blood was drawn.

Urine analyses The urine was transferred, immediately after collection, to a measuring cylinder (containing no preservative), covered with mineral oil, and then immediately analyzed for ascorbic acid as described above. Urea analyses were done according to Van Slyke and Kugel (14).

Plasma analyses Blood was drawn into a syringe and immediately separated into two portions. For the ascorbic acid analysis, 7 cc. were placed in a centrifuge tube containing 14 mgm $\text{K}_2\text{C}_2\text{O}_4$ and quickly centrifuged (5 minutes). Of the plasma, 3 cc. were deproteinized with metaphosphoric acid (15) and immediately centrifuged for 15 minutes. Five cc. samples of the supernatant were titrated with a 2,6-dichlorophenol indophenol solution (approximately 0.0065 gram per 100 cc.). Blank determinations were done with 2 per cent metaphosphoric acid. The indicator was standardized against a 1 cc. sample of ascorbic acid solution (about 10 mgm., accurately weighed, in 200 cc. of 2 per cent HPO_4), plus 4 cc. of 2 per cent HPO_4 . Urea analyses were done on the plasma or serum from another portion of blood (13).

⁴ Glacial acetic acid is more convenient to use, and less expensive than trichloroacetic acid, which has been used in various laboratories (2).

EXPERIMENTAL

The preservation of ascorbic acid in urine. In studying some of the factors involved in the oxidation of ascorbic acid in the average untreated urine over varying periods of time prior to analysis Sendroy and Schultz (2) showed that the oxygen tension was the most important. They were able to retard oxidation by exclusion of air from the sample bottles which were stored over night in the cold. Although their procedure gave satisfactory results it was thought desirable to use a simpler technique, and one which would eliminate the loss of titratable ascorbic acid in the presence of air by inhibition of the action of oxidative catalysts such as copper (16, 17).

Fujita and Iwatake (18) found that 2 per cent metaphosphoric acid greatly retarded spontaneous oxidation of ascorbic acid solutions a fact confirmed by Musulin and King (19). We endeavored to use this material as a protective agent for urinary ascorbic acid. Solid HPO_4 to make a concentration of 2 or 5 per cent in solution was added to urine samples with and without added ascorbic acid. Portions of the urine samples were immediately titrated with 2,6-dichlorophenol indophenol. The remainder was then stored for about 24 hours in the refrigerator in specimen bottles exposed either to one-half volume of air or merely to the bubble left when a completely filled bottle was stoppered. Varying amounts of toluene were also used. After 24 hours the samples were again analyzed for ascorbic acid. The results are indicated in Table I. Apparently under these conditions there is a loss of titratable material equivalent in concentration to from 0 to 3 mgm. of ascorbic acid per 100 cc. of urine. The percentage loss will therefore tend to be greater in the samples of lower concentrations although the variation from urine to urine is such that no rule of prediction may be applied. In general it may be concluded from these results that in the presence of air at low temperature (5°C.) over a period of 24 hours ascorbic acid may be preserved in urine, in the presence of 5 per cent metaphosphoric acid and 0.2 per cent toluene, with a probable loss of 6 per cent.

While we were engaged in the preliminary studies of the effect of metaphosphoric acid, Dr. E. S. G. Barron informed us that he had already found a suitable preservative for urinary ascorbic acid, and kindly suggested its use for our studies which we were anxious to do while the clinical material was available. The inhibitory effect of 8-hydroxyquinoline on ascorbic acid oxidation in vegetable and fruit juices has since been reported by Barron, Barron, and Klemperer (17). The experimental data in support of the use of 8-hydroxyquinoline and sulfuric acid for urine analysis have not as yet been published from their laboratory. However in the mean while, it will suffice for us to say that we have thoroughly tested the preservative that we have found it, with toluene and storage in the cold, more effective than any other procedure in preventing the oxidation of ascorbic acid in the varying concentrations found in urine after a test dose that the loss of titratable ascorbic acid

TABLE I

The preservation of ascorbic acid in urine kept at 5°C. for 24 hours with added metaphosphoric acid

Sample number	Final concentration in urine of added		Amount of air left in specimen bottle	Ascorbic acid concentration			
	HPO ₄	Tol. concn		Initial	Final	Loss	Part of initial preserved
	per cent	per cent		mgm per 100 cc.	mgm per 100 cc.	mgm per 100 cc.	per cent
1	2	0.3	Bubble	28.2	25.3	2.9	89.7
	2	1.0	Half volume		25.1	3.1	89.0
2	2	0.3	Bubble	30.5	29.3	1.2	96.1
	2	1.0	Half volume		30.7	+0.2	100.7
3	2	0.3	Bubble	28.6	26.8	1.8	93.7
	2	1.0	Half volume		26.6	2.0	93.0
4	2	0.3	Bubble	32.3	30.4	1.9	94.1
	2	1.0	Half volume		30.3	2.0	93.8
5	2		Half volume	13.7	13.4	0.3	97.8
	2	1.0	Half volume		13.4	0.3	97.8
	5		Half volume		13.5	0.2	98.6
	5	1.0	Half volume		13.5	0.2	98.6
6	2		Half volume	8.76	7.70	1.06	87.9
	2	1.0	Half volume		7.70	1.06	87.9
	5		Half volume		7.92	0.84	90.4
	5	1.0	Half volume		8.02	0.74	91.6

is usually limited to 2 to 5 per cent over a period of 24 to 48 hours. Moreover we have found that the concentration of preservative may be increased at least five times without affecting the titration. It therefore does not matter if the 750 cc. specimen bottles are not filled to capacity.

Since the urine of nephritic patients is usually albuminous the effect of protein on the titration of ascorbic acid in urine was investigated. For this purpose several 24-hour specimens of urine containing from 0.86 gram to 10.38 grams of protein per liter were collected. Because of the sulfuric acid in the preservative, some protein was already precipitated out when the specimens were received in the laboratory. With stirring this material was uniformly distributed throughout the fluid, which was then divided into three portions. Titrations were done on one portion immediately on another after separation from the solid matter by centrifugation, and on another after deproteinization with metaphosphoric acid (to make a 2 per cent concentration). The results of the titrations of the three portions were practically identical for the several specimens tested. Comparisons of freshly voided albuminous urines with and without deproteinization with trichloroacetic acid failed to show any differences in the titration values.

The preservation of ascorbic acid in plasma. Following Fujita and Iwatake's observation (18) concerning its stabilizing influence in ascorbic acid solutions, it seemed logical to employ metaphosphoric acid for plasma

analyses, since it is also an adequate blood deproteinizing agent, as has been shown by Hiller and Van Slyke (20). When Farmer and Abt's revised procedure was published (15), we had already finished experiments, the results of which were in agreement with their work. In confirmation of their findings, and those of Pijoan, Townsend, and Wilson (21), we observed a loss, on standing, of substance titratable with 2,6-dichlorophenol indophenol. This loss was presumably owing to oxidation of ascorbic acid. On the assumption that such was the case, although no proof exists that it is ascorbic acid that is lost under these conditions, it seemed that the most reliable values were those obtained for samples deproteinized within the minimum time after withdrawal of blood.

In order to prevent such losses of titratable ascorbic acid, Pijoan and Klemperer (22) have recommended as an inhibitor the use of 0.1 per cent KCN in blood before deproteinization. In testing their procedure, we did find increased values as a result of adding KCN to blood, but KCN did not prevent the loss of titratable ascorbic acid which occurred when plasma stood for 2 hours before deproteinization. Furthermore, blank analyses with the protein precipitant and KCN were too high and too variable to afford confidence in the reliability of the results. More recently, others (23, 24) have questioned the efficacy of adding KCN to whole blood when ascorbic acid titrations of metaphosphoric acid filtrates are made with 2,6-dichlorophenol indophenol. The first group of workers (Friedman, *et al.*) found that KCN increased the amount of dye required up to physiological concentrations in plasma. Beyond that, at higher concentrations, KCN filtrates gave lower values than the "unprotected" ones. In all cases, whatever values were obtained remained constant for almost three hours.

Farmer and Abt (24) have come to the conclusion that when KCN is used with their procedure, the inhibitor invalidates results. They found that KCN does not prevent loss of ascorbic acid from blood on standing, although it does decolorize 2,6-dichlorophenol indophenol in HPO_3 solution and in blood plasma filtrates, thereby increasing the amount of ascorbic acid apparently present. Our own results were similar to theirs. It appears, from the foregoing, that KCN, while not preventing loss of ascorbic acid, may, under certain circumstances by its decolorizing effect on the indicator, exhibit a compensating "stabilizing" effect. At the present time, therefore, for dependable ascorbic acid values by the method of Farmer and Abt (15), it would seem best to follow their recommendations (15, 24) that the blood be centrifuged *immediately* after withdrawal, the plasma separated, and deproteinization carried out as quickly as possible.

RESULTS

From the results shown in Table II and Figure 1, there is a distinct, although only roughly quantitative, relationship between the utilization index, as found by urinary excretion studies, and the

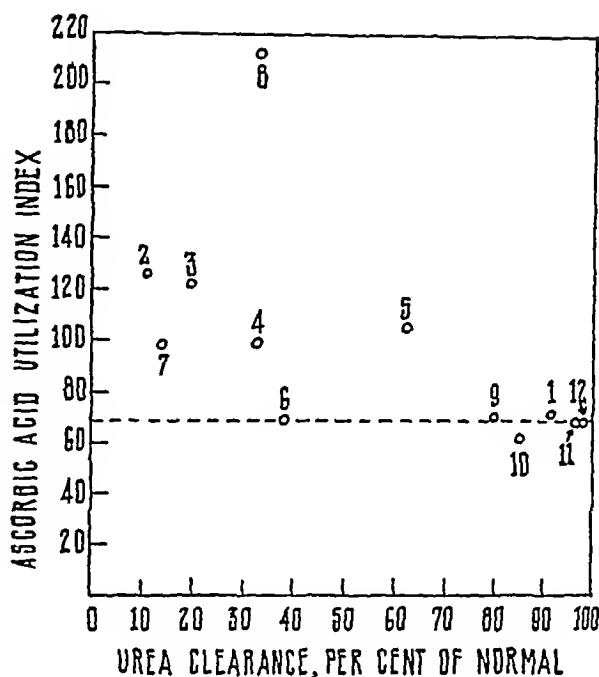


FIG. 1. RELATIONSHIP BETWEEN RESULTS OF URINARY EXCRETION TESTS FOR ASCORBIC ACID, AS INDICATED BY THE ASCORBIC ACID UTILIZATION INDEX, AND KIDNEY FUNCTION, AS INDICATED BY UREA CLEARANCE DETERMINATIONS

The dotted line follows the average, normal value for the index. The numbers of the points refer to the subjects of Table II.

kidney function as judged by the maximum or standard urea clearance. With the exception of Case 6, the subjects with low percentages of normal urea clearance show a subnormal urinary excretion of ascorbic acid. The evidence is sufficient to indicate that under otherwise normal conditions of ascorbic acid intake and output, when there is impairment of renal function, as in these cases of nephritis, there is a corresponding ascorbic acid retention, and a lowered excretion in the urine.

If the ratios of ascorbic acid clearance relative to urea clearance be calculated (Column 14, Table II) there is found a more marked and somewhat more quantitative difference in the renal excretion of ascorbic acid of the two groups, normal and nephritic. When kidney function is approximately normal, the ratio is about 0.5. For the nephritic group, the ratio is about 1.1. The increase in the ratio varies with decrease in renal function. These findings are

TABLE II

Results of tests of ascorbic acid utilization and simultaneous ascorbic acid and urea clearance

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Subject number	Sex	Age	Body weight	Condition	12 hour urine specimen contained	Utilization index	Clearance determinations*						
					Red blood cells		Protein	Plasma analysis		Ascorbic acid clearance UV_c/P	Urea clearance		Clearance ratio: ascorbic acid/urea
								Ascorbic acid	Urea		UV_c/P	Per cent of normal	
		years	kgm.		millions	grams		mgm. per 100 cc.	mgm. per 100 cc.				
1	F	18	52	Latent, degenerative Bright's disease	0.4	2.7	71	1.69	12.2	27.5	68.6	92	0.40
2	F	25	65	Chronic, hemorrhagic Bright's disease	0.8	4.5	126	2.53	33.9	6.7	5.8	11	1.16
3	F	30	58	Chronic, hemorrhagic Bright's disease	0.075	4.0	122	1.63 1.49	37.7 41.7	8.8 16.0	7.4 15.9	18 21	1.19 1.00
4	M	24	60	Chronic, hemorrhagic Bright's disease	0.2	0.9	98	2.46 31.7	34.0 31.7	27.1	22.8 24.2	31 32	1.25 1.12
5	M	24	57	Chronic, hemorrhagic Bright's disease	25.0	1.0	105	2.50 1.93	23.3 22.3	31.8 38.9	31.8 39.7	69 56	1.00 0.98
6	M	19	60	Chronic, hemorrhagic Bright's disease	16.0	3.9	69	1.11 1.22	31.4 31.2	26.6 26.9	25.9 25.3	42 34	1.03 1.06
7	M	24	56	Chronic, hemorrhagic Bright's disease	100.0	3.2	98	2.54 2.49	88.8 89.0	10.3 9.4	9.3 8.5	14 14	1.11 1.11
8	M	51	48	Arteriosclerotic Bright's disease		3.2	212	3.08 3.10	40.1 31.3	18.1 20.5	17.3 24.7	33 33	1.05 0.83
9	M	28	70	Normal			(70)	1.70	13.1	20.8	56.6	80	0.37
10	M	36	66	Normal			(61)	1.71 1.63	11.0 10.5	36.5 28.2	60.7 56.8	81 89	0.60 0.50
11	M	29	70	Normal			(68)	1.86	13.2	44.1	61.9	97	0.71
12	M	29	76	Normal			(68)	1.54	8.8	44.6	73.5	98	0.61

* The absolute values for the clearances are all calculated as UV_c/P . The per cent of normal (Column 13) is calculated by either the standard or maximum urea clearance equation (25) according to the urine volume.

consistent with the observations of Friedman, McGoe, and Ralli (11) and of Ralli, Friedman, and Rubin (12), that the ascorbic acid clearance increases with rising plasma concentration until a limiting value is reached.

Under the conditions of our experiments, *i.e.*, with nephritic subjects in whom the renal lesions were in varying degrees of activity, the excretion of ascorbic acid varied with the functional capacity of the kidneys. Presumably, any disease or condition which reduces renal function, *e.g.*, Addison's disease, would also impair the excretion

of ascorbic acid. In such cases, excretion tests for ascorbic acid would give results which would be false with respect to the nutritional state. We suggest, whenever excretion tests give values indicative of ascorbic acid deficit in the body that the tests be further controlled by adequate renal function measurements.

SUMMARY

Abnormally slow excretion of administered ascorbic acid does not necessarily indicate a low ascorbic acid content of the body when renal

function is low, because renal damage retards excretion, even when no ascorbic acid deficit exists. The effect of lowered kidney function on the ascorbic acid clearance runs approximately parallel to the effect on the urea clearance.

BIBLIOGRAPHY

- 1 Harris, L. J., Ray, S. N., and Ward, A., The excretion of Vitamin C in human urine and its dependence on the dietary intake. *Biochem. J.*, 1933, 27, 2011
- 2 Sendroy, J., Jr., and Schultz, M. P., Studies of ascorbic acid and rheumatic fever. I. Quantitative index of ascorbic acid utilization in human beings and its application to the study of rheumatic fever. *J. Clin. Invest.*, 1936, 15, 369
- 3 Drigalski, W. v., Über Vitamin C im Urin Von Gesunden und Kranken. *Klin. Wchnschr.*, 1935, 14, 338
- 4 Siwe, S. A., Das Verhalten des C-Vitamins bei Morbus Addisoni. *Klin. Wchnschr.*, 1935, 14, 1311
- 5 Wilkinson, J. F., Manch, M. D., and Ashford, C. A., Vitamin C deficiency in Addison's disease. *Lancet*, 1936, 2, 967
- 6 Van Eekelen, M., On the amount of ascorbic acid in blood and urine. The daily human requirements for ascorbic acid. *Biochem. J.*, 1936, 30, 2291
- 7 Lund, H., Eine quantitative und spezifische Methode zur Ascorbinsäuretitration im Harn und zur Bestimmung des Schwellenwertes. *Klin. Wchnschr.*, 1937, 16, 1085
- 8 Faulkner, J. M., and Taylor, F. H. L., Observations on the renal threshold for ascorbic acid in man. *J. Clin. Invest.*, 1938, 17, 69
- 9 Wright, I. S., Lihenfeld, A., and MacLenathen, E., Determination of vitamin C saturation. A five-hour test after an intravenous test dose. *Arch. Int. Med.*, 1937, 60, 264
- 10 Wright, I. S., and MacLenathen, E., Vitamin C saturation—Kidney retention after an intravenous test dose of ascorbic acid. *Proc. Soc. Exper. Biol. and Med.*, 1938, 38, 55
- 11 Friedman, G. J., McGoey, C., and Ralli, E. P., The clearance of Vitamin C by the human kidney. *Am. J. Physiol. (Proc.)*, 1938, 123, 71
- 12 Ralli, E. P., Friedman, G. J., and Rubin, S. H., The mechanism of Vitamin C excretion in man studied by simultaneous Vitamin C and inulin clearances. *J. Clin. Invest. (Proc.)*, 1938, 17, 504
- 13 Van Slyke, D. D., Page, I. H., Hiller, A., and Kirk, E., Studies of urea excretion. IX. Comparison of urea clearances calculated from the excretion of urea, of urea plus ammonia and of nitrogen determinable by hypobromite. *J. Clin. Invest.*, 1935, 14, 901
- 14 Van Slyke, D. D., and Kugel, V. H., Improvements in manometric micro-Kjeldahl and blood urea methods. *J. Biol. Chem.*, 1933, 102, 489
- 15 Farmer, C. J., and Abt, A. F., Determination of reduced ascorbic acid in small amounts of blood. *Proc. Soc. Exper. Biol. and Med.*, 1936, 34, 146
- 16 Barron, E. S. G., De Meo, R. H., and Klemperer, F., Studies on biological oxidations. V. Copper and hemochromogens as catalysts for the oxidation of ascorbic acid. The mechanism of the oxidation. *J. Biol. Chem.*, 1936, 112, 625
- 17 Barron, E. S. G., Barron, A. G., and Klemperer, F., Studies on biological oxidation. VII. The oxidation of ascorbic acid in biological fluids. *J. Biol. Chem.*, 1936, 116, 563
- 18 Fujita, A., and Iwatake, D., Über die Bestimmung von Vitamin C mittels 2,6-Dichlorphenolindophenol. *Biochem. Ztschr.*, 1935, 277, 293
- 19 Musulin, R. R., and King, C. G., Metaphosphoric acid in the extraction of Vitamin C. *J. Biol. Chem.*, 1936, 116, 409
- 20 Hiller, A., and Van Slyke, D. D., A study of certain protein precipitants. *J. Biol. Chem.*, 1922, 53, 253
- 21 Pijoan, M., Townsend, S. A., and Wilson, A., Determination of reduced ascorbic acid in blood. *Proc. Soc. Exper. Biol. and Med.*, 1936, 35, 224
- 22 Pijoan, M., and Klemperer, F., Determination of blood ascorbic acid. *J. Clin. Invest.*, 1937, 16, 443
- 23 Friedman, G. J., Rubin, S. H., and Kees, W., Effect of addition of KCN to whole blood on indophenol-reducing power of plasma. *Proc. Soc. Exper. Biol. and Med.*, 1938, 38, 358
- 24 Farmer, C. J., and Abt, A. F., Invalidation of plasma ascorbic acid values by use of potassium cyanide. *Proc. Soc. Exper. Biol. and Med.*, 1938, 38, 399
- 25 Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry Vol. I Interpretations*. Williams and Wilkins Co., Baltimore, 1931, p. 335

THE SIGNIFICANCE OF PROLONGED STREPTOCOCCAL ANTIBODY DEVELOPMENT IN RHEUMATIC FEVER¹

By ALVIN F. COBURN AND RUTH H. PAULI

(From the Department of Medicine, College of Physicians and Surgeons, Columbia University and the Presbyterian Hospital, New York City)

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It has been shown that patients with rheumatic fever continue to produce antibodies to hemolytic streptococcus for months after the subsidence of an apparently transient respiratory infection. The various clinical types of rheumatic fever are accompanied by characteristic types of antibody response. In monocyclic attacks with acute onset, a period of severe illness and relatively rapid recovery, the titer reaches maximum relatively early—within a month of the onset of rheumatic symptoms. In attacks with insidious onset, where repeated cycles of progressive severity culminate in severe carditis months later, the titer rises more gradually, reaching its maximum height late in the illness (1). This close relation between the course of antibody production and the clinical character of the rheumatic attack has stimulated us to investigate the underlying reason for the prolonged development of streptococcal antibody in this disease.

The simple hypothesis that the infectious agent persists throughout the period of rising titer does not lend itself to direct proof or disproof in the human subject. During convalescence from pharyngitis, hemolytic streptococcus commonly disappears from the mucosa of the upper respiratory tract, and only the occasional patient furnishes the opportunity to recover it later from deeper tissues, such as an accessory sinus, middle ear or tonsils. The impossibility of thorough bacteriological studies of deep tissues in human subjects has obliged us to use an indirect approach to explain the significance of prolonged antibody development. The purpose of this paper is to summarize such indirect evidence as we have been able to obtain and to indicate its bearing on the rheumatic fever problem. The data have been collected from naturally occurring throat infections in human subjects and from experimental infections in the guinea pig.

¹ The work reported in this communication was carried out under the W. K. Kellogg Foundation Fund.

I. THE PROLONGED DEVELOPMENT OF BOTH ANTISTREPTOLYSIN AND ANTI-M SUBSTANCE

Quantitative studies have shown that the titer of antistreptolysin rises following uncomplicated streptococcal throat infections, reaching a maximum value in about three weeks following infection.² During continued rheumatic activity, however, this titer increases progressively for extended periods of time, sometimes as much as six months. The titer does not fall until recovery has begun. This is illustrated (Figure 1) by Patient E. G. who developed different types of rheumatic attacks in successive years (1934 intense monocyclic, 1935 insidious polycyclic).

Patient E. G., Number 345168 a six year old girl with rheumatic heart disease, was convalescing at the Pelham Home when in April 1934 she contracted pharyngitis with hemolytic streptococcus Group A, Type 26. Two weeks later she developed a severe attack of rheumatic fever, reaching maximum intensity one month after infection. At this time she had pancarditis and pulmonary consolidation. At the height of illness the antistreptolysin titer reached its peak, about five weeks after infection.

One year later this patient again contracted pharyngitis with hemolytic streptococcus Group A (not typed). Two weeks after infection she complained of fatigue and vague abdominal pain (Cycle 1 of rheumatism). Five weeks after infection she developed chest pain, slight erythema marginatum and tachycardia (Cycle 2) although these symptoms were mild and the blood sedimentation rate rose to 100 mm. per hour (Westergren). Five weeks after infection she developed severe precordial pain, polyarthritides and facial edema. This was the third cycle in the rheumatic attack but the first with frank clinical symptoms. There were four other classical cycles in the next four months. In contrast to the previous year the antistreptolysin titer rose slowly during this attack and did not reach its peak until about five to six months after infection.

This patient illustrates a characteristic relationship be-

² Observations in the past have shown that other infections, including pneumonia, chickenpox, measles, mumps, common colds, influenza, appendicitis, and non-specific agents (such as typhoid vaccine and tetanus antitoxin) do not increase the antistreptolysin

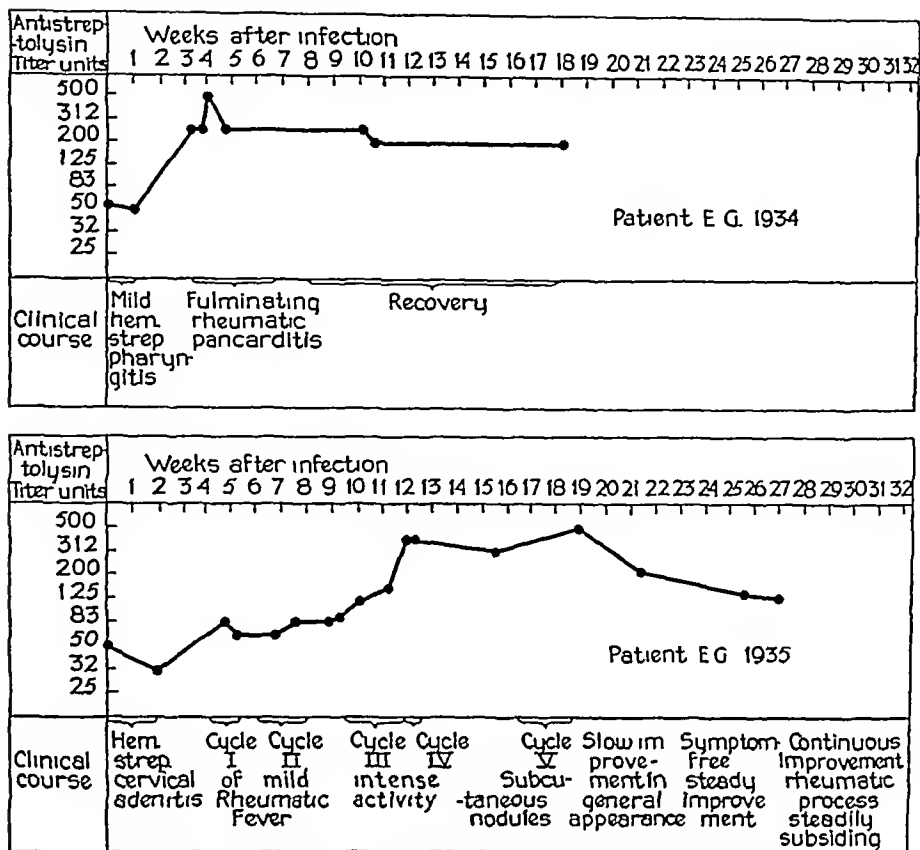


FIG 1 THE ANTISTREPTOLYSIN CURVES ASSOCIATED WITH DIFFERENT TYPES OF RHEUMATIC ATTACKS

tween titer curve and clinical course severe, acute attack accompanied by early peak in antistreptolysin titer, prolonged polycyclic attack accompanied by a slowly rising titer reaching its peak several months after infection.

The presumable stimulus for this progressive increase in antistreptolysin is the continuous release of streptolysin into the tissues of the patient. If the release of streptolysin should occur alone, without other streptococcal antigens, streptolysin then would appear to be closely associated with the pathogenesis of rheumatic fever. However, if the prolonged development of antistreptolysin is accompanied by a similar development of other streptococcal antibodies, then the antistreptolysin response merely indicates that the infectious agent as a whole remains active somewhere in inaccessible tissues. To obtain evidence bearing on these possibilities, parallel studies were made of the development of antistreptolysin and of precipitins to Lancefield's type-specific M substance.

Anti-M precipitins are known to occur in non-rheumatic subjects following hemolytic streptococcal throat infections (2, 3). They generally appear and subside a little later than antistreptolysin, being maximal at about 5 weeks after the onset of infection (3). Anti-M precipitins also occur in the sera of rheumatic patients, but we have not followed the course of their development in relation to antistreptolysin except in a few isolated cases (3). We therefore conducted the following study.

Procedure

Sera were collected from rheumatic subjects at weekly intervals from the onset of pharyngitis over a period of three to six months. Sixteen individuals who developed rheumatic fever comprise the group reported in this study. Each sample of serum was tested in three ways. Antistreptolysin was titrated according to our standard procedure (4). Anti-M type-specific precipitins were estimated according to Lancefield's method (5). Type-specific agglutinins were estimated according to the slide agglutination technique originally described by Griffith.

(6) and used by ourselves (7) for the typing of hemolytic streptococcus. Type-specific M-substances for the precipitin tests were prepared by extracting cultures of the organism recovered from the patient's throat during the acute infection. The slide agglutination tests were carried out on the patient's serum with suspensions of the patient's organism and with control suspensions of other serological types

Results

All sixteen patients developed a striking rise in the antistreptolysin titer and all developed type-specific antibodies. Fourteen showed precipitins and eleven gave slide agglutination reactions. The times when maximum titers occurred are shown in Table I

TABLE I

Time of occurrence in the rheumatic attack of the patient's maximum antibody level

Patient	Week of rheumatic attack			Clinical condition
	Antistreptolysin	Anti M		
		Precipitin	Agglutinin	
A.L	3	2	2	Height of attack
A.V	10	18	24	Early subsidence
G.E	3	7		Height of attack
B.D	3	12	7	Early subsidence
J.H	3	6	2	Height of attack
D.K	3	5	5	Early subsidence
A.N	10	12		Subsiding
M.V	5	3		Subsiding
V.O	4	9	9	Early subsidence
G.P	3	2	1	Early subsidence
M.We	1	2		Height of attack
M.Wi	5	8	8	Convalescing
G.B	1	3		Height of attack
H.D (a)	1	2	2	Height of attack
H.D (b)	6	6	6	Height of attack
B.E	6	8	8	Subsiding

In the majority of cases antibody to the M-substances continued to increase after the antistreptolysin titer had reached its maximum level. Hence any prolongation of the immune response in these patients affected both antibodies and not antistreptolysin alone. These findings point to the prolonged activity of hemolytic streptococcus as a whole.

II THE EFFECT OF POSTSTREPTOCOCCAL SEPTIC COMPLICATIONS ON ANTIBODY PRODUCTION

Preliminary observations on non rheumatic subjects (3) indicated that in the absence of com-

plications, maximum antistreptolysin titers were usually reached within three weeks after the onset of scarlet fever and pharyngitis. Subsequent studies have been made of a larger group of patients during recovery from streptococcal respiratory infections. Some of these had normal recoveries and others developed septic complications.

Eighty five cases, including fifty-four of scarlet fever and thirty-one of pharyngitis, were studied from the onset of infection through convalescence for periods varying from six to sixteen weeks. Of fifty nine patients who had normal recoveries, fifty seven reached their maximum titer within a month and two were delayed beyond a month. Twenty six patients had complications of varying degrees of severity. Of these, only fourteen reached maximum titer within a month. The data for patients with and without complications are presented in Table II

TABLE II

The number of patients reaching their maximum antistreptolysin titer at various times after the onset of streptococcal throat infections

Patients with	Weeks after onset of infection				
	1	2	3	4	Later
Uncomplicated recoveries (59)	12	19	17	9	2
Septic complications (26)	2	5	4	3	12

The development of a septic complication was followed in most cases by a further rise in antistreptolysin titer and the time of this rise depended on when the complication appeared. Late increases in antibody titer occurred in fourteen patients. Twelve of these late rises were associated with demonstrable active infection.

In summary, it appeared that the antistreptolysin titer increased as long as the infectious agent continued to be active.

III THE EXPERIMENTAL PRODUCTION OF A PROLONGED ANTISTREPTOLYSIN RESPONSE

It has been possible to produce an increase in antistreptolysin titer in guinea pigs by infecting them experimentally with Group A hemolytic streptococcus, NY 5. Using the agar focus technique, McBroom in this laboratory induced

the development of antistreptolysin titers ranging from 50 to 1000 units in 33 out of 37 animals (Uninfected controls had titers of 0 to 10 units) The agar foci gave rise to abscesses which ruptured spontaneously and healed, as a rule, within 6 weeks With healing, the titers either remained at plateau level without further rise for a number of weeks, or fell rapidly to zero

Attempts were made to raise the titers of these guinea pigs still further by the injection of various nonspecific antigens Filtrates from *B typhosus*, *B Leptisepticus*, and meningococcus were administered either intravenously, intrapericardially, subcutaneously, or intracutaneously None of these materials had any effect on the antistreptolysin titer levels

It was possible, however, to induce secondary rises in titer more than two months after the original focus, by the introduction of a new agar focus after the healing of the primary lesion Fifteen guinea pigs were treated in this way, eight receiving the new focus before and seven receiving it after the titer induced by the first infection had subsided Thirteen of these animals developed higher antistreptolysin titers after the healing of the second focus than they had shown after the first Of the seven pigs whose second focus was given after the antibody titer to the first had subsided, six showed a greater response to the second focus than they had to the first Five pigs received a third focus, which in each case gave rise to a new increase in titer The effect of repeated foci on the antistreptolysin titers of guinea pigs is shown in Figure 2

In summary, the progressive development of high antistreptolysin levels in the guinea pig appeared dependent upon repeated infections with hemolytic streptococcus

IV THE RELATION OF INFECTION TO THE ANTISTREPTOLYSIN TITER CURVE IN GUINEA PIGS

Another group of 25 pigs was given agar foci and sacrificed at intervals thereafter Bleedings were taken from the heart every three weeks for antistreptolysin determinations Six pigs with suppurative lesions and six others with healed foci were killed during the period of rising titers (before 6 weeks) The remaining thirteen pigs were autopsied at the end of 2 months or later,

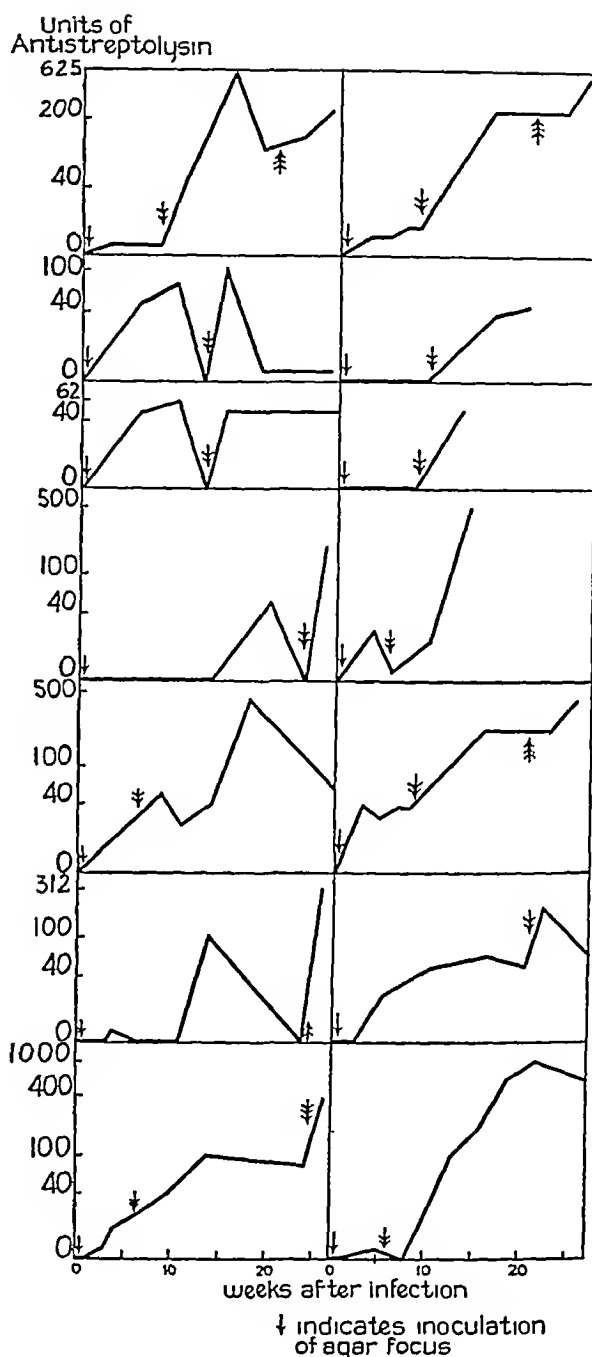


FIG 2 ANTISTREPTOLYSIN CURVES IN GUINEA PIGS FOLLOWING THE INDUCTION OF STREPTOCOCCAL ABSCESSES

when their foci had healed and their titers had reached a maximum Careful bacteriological studies were made from the site of the focus, heart's blood, lymph nodes, spleen, liver, and kidney

The results of these cultures were as follows. No organisms were recovered from guinea pigs with stationary or falling titers irrespective of when the animals were killed. The twelve pigs killed during the period of rising titer had viable organisms in the depths of the foci.

SUMMARY

Findings are presented which support the view that prolonged increases in streptococcal antibody, such as are observed in rheumatic fever, signify subclinical activity of hemolytic streptococcus

The authors are indebted to Drs. Josephine McBroom and Samuel Hunt for their help in the animal experiments.

BIBLIOGRAPHY

- 1 Coburn, A. F., and Pauli, R. H., Studies on the immune response of the rheumatic subject and its

relationship to activity of the rheumatic process. *J. Clin. Invest.* 1935 14 769

Specific and non-specific changes in blood protein during acute rheumatism with carditis. *Internat. Clin.* 1936 4 49

- 2 Swift, H. F., and Hodge, B. E., Type-specific anti M precipitins in rheumatic and non rheumatic patients with hemolytic streptococcal infections. *Proc. Soc. Exper. Biol. and Med.*, 1936 34 849
- 3 Coburn, A. F., Observations on the mechanism of rheumatic fever. *Lancet*, 1936 2 1025
- 4 Coburn, A. F., and Pauli, R. H., Studies on the immune response of the rheumatic subject and its relationship to activity of the rheumatic process. *J. Exper. Med.*, 1935 62, 129
- 5 Lancefield, R. C., The antigenic complex of *Streptococcus haemolyticus*. *J. Exper. Med.*, 1928 47 91
- 6 Griffith, F., The serological classification of *Streptococcus pyogenes*. *J. Hyg.*, 1935 34 542
- 7 Pauli, R. H., and Coburn, A. F., Studies on the serological typing of *Streptococcus haemolyticus*. *J. Exper. Med.*, 1937 65 595

THE PROPHYLACTIC USE OF SULFANILAMIDE IN STREPTOCOCCAL RESPIRATORY INFECTIONS, WITH ESPECIAL REFERENCE TO RHEUMATIC FEVER¹

By ALVIN F. COBURN AND LUCILE V. MOORE

(From the Department of Medicine, College of Physicians and Surgeons, Columbia University and the Presbyterian Hospital, New York City)

(Received for publication October 11, 1938)

The close association of rheumatic fever with hemolytic streptococcal infections has led several investigators to test the applicability of sulfanilamide to the treatment of this disease. The results (1, 2) have indicated that the drug is of no therapeutic value in acute rheumatism. In fact, unlike those diseases which merely fail to respond to sulfanilamide therapy, rheumatic fever is definitely aggravated by the drug. This is not surprising if one considers the unusual sensitivity of patients with active rheumatism to streptococcal products.

Our observations over the past ten years have shown that rheumatic subjects who escaped hemolytic streptococcal infections also escaped rheumatic fever. It therefore seemed possible that the development of rheumatic attacks might be avoided if streptococcal respiratory infections could be prevented. With this objective in mind, we have conducted studies in the prophylaxis of hemolytic streptococcal infections during the past two years. The data to be reported comprise experiments on guinea pigs and observations on rheumatic subjects.

EXPERIMENTS ON GUINEA PIGS

Streptococcal cervical lymphadenitis or lumps, an epizootic disease originally described by Smith (3), was selected as a close parallel in the guinea pig to pharyngitis in the human subject. The prophylactic effect of sulfanilamide was therefore tested on both spontaneous and induced infections of this character.

EXPERIMENTAL PROCEDURE

1. Spontaneous infections were obtained in healthy guinea pigs weighing about 250 grams during the winter months by placing one infected animal in each of two groups of 15 normals. To equalize the virulence of in-

¹ The work reported in this communication was carried out under the W. K. Kellogg Foundation Fund.

fection to which the healthy pigs were exposed, the infected animals (kindly furnished by Dr. C. V. Seastone of the Rockefeller Institute, Princeton) were alternated daily between the two groups, one of which received sulfanilamide, the other not.

2. *Induced infections.* In order to avoid the delay involved in the spontaneous transmission of infection through a colony a matter of many weeks, more severe infections were induced by the introduction of 0.2 cc. of an 18-hour culture of hemolytic streptococcus Group C, Seastone's strain, Number L7628 into the nares of each pig. The second and third colonies were infected in this way.

3. Sulfanilamide was administered by hypodermoclysis. In the course of 24 hours each animal received 0.15 gram in the form of a 1 per cent solution of the drug in normal saline. Doses were administered every eight hours. This amount of medication maintained a concentration in heart's blood of 80 to 100 gamma per cc.

4. All animals were examined daily for signs of lymphadenitis.

5. Bacteriological studies were made on pigs which died and on those which were killed. The tissues cultured were lymph glands, heart's blood, liver, spleen, and lung. Cultures were made in broth and on blood agar plates incubated under both aerobic and anaerobic conditions.

6. Histological studies were made from fixed preparations of the infected foci as well as from lymph glands, lung, liver, spleen, and kidney.

The first colony of 30 animals exposed to infection by contact with diseased guinea pigs was divided into two groups: (a) 15 treated with sulfanilamide and (b) 15 controls. The period of exposure was 7 weeks. At the end of this time all 15 of the untreated controls had developed cervical adenitis and 13 yielded hemolytic streptococcus Group C. At the same time, none of the treated pigs had palpable cervical glands. At autopsy the cervical nodes of 6 animals were slightly larger than normal. Cultures of the entire treated group were negative for hemolytic streptococcus. Although these experiments differed considerably in detail from those of Seastone (4), our observations are in good agreement with his.

The second colony of 24 pigs, infected by the introduction of 0.2 cc of culture in the nares, was divided into two groups, (a) 12 treated with sulfanilamide before and after insufflation of culture, and (b) 12 untreated controls. Within three weeks all controls developed marked enlargement of the cervical glands from each of which hemolytic streptococcus Group C was cultured. The treated animals appeared to be normal. They were killed at this time and on autopsy the cervical glands were found to be slightly enlarged in all pigs. However, hemolytic streptococcus Group C was isolated from only 4 of the 12 pigs.

The occurrence of slight cervical enlargement and the absence of organisms on culture or smear in 8 animals suggested that organisms might be present in the glands and be controlled by the bacteriostatic action of sulfanilamide. The following experiment investigated this possibility. A third colony of 24 pigs were all treated with sulfanilamide before and after infection by the nasal route. After 14 days chemotherapy was discontinued. Half of the animals were killed (Group 1). Their cervical glands were removed under sterile precautions, ground, and injected into the abdomen of 12 normal pigs (Group 3). The remaining 12 pigs (Group 2) were kept without further medication.

One month later Groups 2 and 3 were sacrificed. The animals in Group 3 were all found to be normal and all cultures were sterile. Hemolytic streptococcus was recovered from 4 out of 12 pigs in Group 2.

In summary, sulfanilamide appeared to give complete protection to a colony of guinea pigs against spontaneous lymphadenitis with Group C hemolytic streptococcus. Sulfanilamide seemed to protect about two-thirds of the animals which received a large dose of the same organisms intranasally, and modified the disease in the remaining third.

CLINICAL STUDIES

Study I

Limited observations (5) had suggested that the incidence of septic complications following streptococcal infections might be diminished by sulfanilamide therapy during the infections. We

therefore investigated the effect of sulfanilamide, administered during pharyngitis, on the incidence of rheumatic recrudescences.

The patients were selected from a group of rheumatic subjects who have been under our observation for a number of years. Each hemolytic streptococcal throat infection that was seen by us within 24 hours of the onset of symptoms was treated for 5 to 14 days with sulfanilamide. Patients who contracted similar infections but did not report their symptoms until after 24 hours were used as controls. Careful observations were made on a group of 40 children and young adults. The results are summarized in Table I.

TABLE 1

The effect of sulfanilamide administered during pharyngitis on the incidence of rheumatic recrudescences in rheumatic subjects

Treated with sulfanilamide		Not treated with sulfanilamide	
Patient	Severity of rheumatic attack	Patient	Severity of rheumatic attack
Bar	++	Cal	0
Ben	+++	Con	0
Bou	+	Doy	0
Dig	0	Dro	+++
Ear	++++	Fay	+
Kea	0	Fro	0
Kel	+	Gra	0
Kon	±	Kie	+++
Kor	0	Lyn	0
Luo	++	Mal	++++
Met	++	A Ma	0
Men	0	T Ma	++
Noo	0	Nei	+++
Phi	+++	D O B	+
F Ry	±	Pag	++++
Sav	±	San	+++
Sch	0	Sol	0
Spe	0	Ste	+
Wal	+	Wal	+
Win	+	Zel	0
Total number of cases 20		Total number of cases 20	
Fulminating	1	Fulminating	2
Severe	2	Severe	4
Moderate	2	Moderate	1
Mild	5	Mild	4
Questionable	3	Questionable	0
Escaped	7	Escaped	9

From Table I it is seen that 13 treated cases² and 11 untreated cases developed rheumatic

² The antistreptolysin responses of these patients were similar to those which occurred in untreated rheumatic subjects who developed attacks.

manifestations. The latter were possibly a little more severe. Sulfanilamide therapy begun after the onset of pharyngitis appeared to have no effect as a prophylactic agent in preventing rheumatic recrudescences. These findings are in accord with the observations of Massell (1).

The efficacy of sulfanilamide in preventing guinea pigs from contracting spontaneous Group C infections suggested that it might also be of use in preventing the occurrence of Group A respiratory infections in human subjects. To investigate this possibility three additional clinical studies were carried out.

Study 2

Procedure The patients were 29 rheumatic girls between the ages of 6 and 14 who were convalescing at the Pelham Home. The majority were quiescent, a few had evidences of smouldering rheumatism. Except for monthly visits from relatives their contacts were limited to the personnel of Pelham Home.* No new patients were admitted to the Home during the experiment, which ran from January 9 to June 17 1937.

Each patient was given by mouth approximately 2 grams of sulfanilamide daily divided into three doses distributed as evenly as possible throughout the 24 hours. Medication was maintained for the whole period of observation. Previous experience (5) had shown that drug symptoms occurred less frequently in children than in adults and did not develop later than two weeks from the start of therapy.

Two throat cultures were obtained from each patient once a week. In addition, cultures were taken of all patients at the onset of any respiratory infection.

Blood sedimentation rates were determined twice a month. Urinalysis and blood counts were done several times during the course of the experiment.

A careful clinical history was taken daily and a physical examination made once a week.

Results No patients developed any drug manifestations. A few showed slight anorexia and failed to gain weight. There was no evidence of blood destruction or renal irritation.

Hemolytic streptococcus was present in the throat flora of 6 patients at the time medication was started. Two of these were carriers and continued to harbor the organism throughout most of the period. The other 4 became negative within a month after the drug was started. Hemolytic streptococcus appeared briefly in the

throat flora of one other patient at about the middle of the period of observation.

The clinical results may be described as follows: (a) Four patients who already had hemolytic streptococcus in the throat flora at the start showed brief mild rheumatic recrudescences within a few days after starting sulfanilamide. (b) Six patients experienced vague symptoms. It was impossible to know whether these were related to rheumatic activity. (c) Nineteen patients were entirely symptom-free throughout the period.

The blood sedimentation rates remained normal in all but 5 individuals. Four of these rises were transient, occurring in the patients who developed rheumatic symptoms on starting the drug. The fifth occurred during a severe attack of bronchitis.

In summary during the course of the study there were no drug symptoms, no frank streptococcal infections and no attacks of acute rheumatism. Sulfanilamide did not prevent the occurrence of a number of 'common colds,' one pneumococcus ear infection, and one severe bronchitis of unknown etiology.

Study 3

The findings in Study 2 again suggested that sulfanilamide might be of prophylactic value in streptococcal respiratory infections. The third study was planned to obtain critical information on a group of rheumatic children known to be exposed to hemolytic streptococcal infection. In the foregoing study which was performed in a closed colony, there was no indication that the patients had contact with streptococcal infection other than their association with two known carriers of this organism. In the course of Study 3, 4 new patients were admitted to The Pelham Home at different times. One of these was recovering from pharyngitis, another from tonsillectomy, and the other two had acute rheumatism.

Procedure The general procedure was the same as in Study 2, with certain exceptions, as follows:

(a) Six patients from whom sulfanilamide was withheld for various reasons served as untreated controls.

(b) The dosage level was raised gradually over the first 2 weeks to approximately 2 grams per patient per day.

* The Pelham Home is a convalescent home caring for 30 girls with rheumatic heart disease. The personnel comprises a teacher, 3 nurses, and 3 domestics.

(c) The concentration of sulfanilamide in the blood was determined every two weeks. Blood samples were collected at various times after medication to include diurnal fluctuations.

Results As in Study 2, no patient developed drug manifestations, but failure to gain weight was noted in a few instances.

The blood sulfanilamide concentration remained fairly constant after the maintenance dosage was established. The average blood concentration for all patients varied between 29 and 55 gammas per cc with a general average of 39 gammas for the whole group. The individual data are presented in Table II.

TABLE II

The concentration of sulfanilamide expressed as gammas per cc in the blood of rheumatic children kept on maintenance medication at The Pelham Home from November 1937 to June 1938

Patient	Weight	Dose	Nov	Dec	Jan.	Feb	Mar	Apr	May	June	Average
	lbs	grams									
Ava	63	2	18 38	30 32	55 40	40	33 35	60 35	35 55	50	39
Cap	100	2	8 18	30 25	30 35	40	28 30	40 20	25 45	35	31
Cla	78	2	8	10 15	33 40	35 35	35 40	50 38	40 35	45	34
Edu	78	2	18	30 62	25 35	40 38	35 32	60 37	40 45	50	40
Fah		2	20 58	50 56	30 35						42
Gor		2	10 20	30 32	30 35	40 40	38 43	40 35	50 40	45	39
Gre	46	2	18 54	54 55	35 55	55 70	55 60	50 35	45 35	60	51
Hob	102	2	10	30 45	18 30	35	28	25 40	40 35	35	33
Iva	78	2	15	36 62	25 40	30 40	40 33	55 45	55	50	42
Lil	108	2	10 34	44 30	40 40	40	35 35	20 40	40 50	50	38
Lil	62	2	22 38	40 35	55 40	40	14 35	45 50	45 70	43	
Max	50	2	22 42	60 63	33 48	37	40 50	70 55	55 60	35	50
Mor	100	3	10 18	26 18	30 55	55 40	35 35	33 40	60 45	50	40
O'Br	67	2	24 22	36 35	40 50	35 40	5 38	50 40	55 40	50	35
Rio	79	2	8	32 45	45 40	40 32	33 35	30 40			37
Ros	100	2	12 36	20 25	35	20 20	28 32	40 20	40 35	35	29
Sal	110	2	14	28 45	20	30 30	20 35	55 35	30 35	40	34
San											
San	53	2	20	38 55	40 35	55 55	50 40	50 35	35 50	40	46
Sea	88	2	6 28	28 20	40	25 25	25 33	40 40	35	15	29
Spe	106	2		30 35	30 30	30 30	30 25	40 30	40 25	30	31
Ste	47	2	15	38 60	50 35	50 60	55 55	55 55	65 55	55	55
Tax	87	2	10	40 53	30	35 35	30 35	40 35	40 55	45	40
Vol	98	2	14 32	40	20 35	35 33	30 22	40 30	30 35	35	32
Wei	67	2	8	30 52	30	35 40	40 32	40 35	45 40	15	35
Patients (total number) 25											39

Altogether 26 patients received sulfanilamide for considerable periods. At the start of the study, hemolytic streptococcus was present in the throat flora of 2 patients. One (Rio) was a carrier who remained positive throughout. The other (Kov) was recovering from pharyngitis and soon became negative. Of 25 other patients whose throat flora were negative at the start of the study and who received sulfanilamide, in only 2 was it possible to find a positive culture of hemolytic streptococcus at any time. In one of

these the organism appeared for 48 hours in moderately large numbers, coincident with respiratory symptoms (Vol). In the other, 3 or 4 colonies were isolated from each of 3 widely spaced cultures. They were not accompanied by symptoms of any sort.

Of the 6 unmedicated patients, 4 had hemolytic streptococcus in the throat flora on one or more occasions. These data are summarized in Table III.

The clinical results showed that 25 out of the 26 medicated patients remained free of rheumatic activity. One patient developed changes which could be interpreted as active rheumatism. This girl (Vol) had a respiratory infection and cervical adenitis and maintained a high blood sedimentation rate over a period of several months in the absence of fever, tachycardia, rise in antistreptolysin titer, and rheumatic symptoms. It seems wisest to classify this case as rheumatic activity, although it was not possible to make a definite diagnosis.

The absence of rheumatic activity in patients receiving sulfanilamide is well illustrated by the sedimentation rates listed in Table IV.

In summary, these observations showed that quiescent rheumatic subjects can be maintained for many months at a high blood sulfanilamide level without demonstrable ill effects. These patients were visited by their families, were exposed to 2 patients with streptococcal infections who were introduced into The Pelham Home, and lived in close proximity with a carrier of hemolytic streptococcus and with 6 non-medicated patients who had positive cultures or rheumatic activity or both at some time during the experiment. Only one of the 26 medicated patients contracted an infection with hemolytic streptococcus in the throat flora. Twenty-five highly susceptible rheumatic children were maintained in good health throughout the winter and spring months.

Study 4

Although the patients in Study 3 were living in close contact with one another in a small institution, nevertheless their general environment with respect to nutrition, housing, and rest facilities was much better than that in which the average rheumatic child in New York City lives.

TABLE III

The occurrence of hemolytic streptococcus in the throat flora of rheumatic children at The Pelham Home from October 1937 through June 1938

Patient	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Comments
RECEIVED SULFANILAMIDE										
Ava.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Cap.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Cl.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Edw.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Fah.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	Moved to Virginia Jan. 20
Gor.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Gre.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Hob.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Iva.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Kov.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	Admitted with tonsillitis within a week after start of medication of other patients—Sulfanilamide begun at once
Lib.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Lil.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Max.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Mor.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
O'Br.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Rio.	0 0 0 0	0 0 0 0 0	0 0 +	+ + 0 0 +	+ 0 0	0 0 + +	+ + 0 0	0 0 0 0	0 0 0 0 0	Carrier. Went home to New York City April 20
Ros.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Sal.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
San.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
San.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
San.	0 0 0 0	0 0 + 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Spe.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	Admitted January 1
Ste.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Tag.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Vol.	+ 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Wel.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
UNVACCINATED										
Coc.										
Far.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	Admitted May 1 with severe rheumatism
McG.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Roe.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
San.	0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	
Toe.	+ 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0 0	Left Pelham Home Nov. 30 for tonsillectomy. Readmitted Feb. 1 during convalescence

TABLE IV

The blood sedimentation rates (Westergren) of rheumatic subjects kept on maintenance medication with sulfanilamide at The Pelham Home from October 1937 through June 1938

Patient	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Ava.	14	15 14	11 8	7 7	15 5	5 5	6 0	12 12
Cap.	10	7 8	7 7	7 7	4 5	4 4	4 4	4 10
Cl.		6 7 20	7 22	10 5	5 5	4 20	4 9	5
Edw.	0 6	5 5	5 5	5 8	2 4	5 8	4 5	4 3
Fah.	5	5 15	4 5	3 20				
Gor.		20 10	8 12	9 15	0 24	7 12	7 7	6 7
Gre.	9	10 10	10 17	12 10	4 6	8 5	7 5	6 7
Hob.	6	4 4	7 9	4 4	4 6	7 15	5 3	7 8
Iva.	7	9 4	8 4	5 11	7 4	5 3	3 3	
Kov.		10 15	7 8	3 12	4 5	5 3	5 3	
Lib.		14 19	8 8	14 11	8 7	10 0	14 8	7 14
Lil.	12	7 4	5 5	4 4	4 4	5 27	3 10	11 15
Max.	26	20 17	9 15	10 15	12 17	8 10	12 12	12 11
Mor.	14	0 0	12 15	10 12	8 12	10 4	10 9	9
O'Br.	13	17 23	14 9	8 8	8 0	7 7	7 9	
Rio.	18	15 10	15 15	14 15	7 15	7 7	8 13	
Ros.	10	12 5	7 7	10 13	4 14	5 28	7 10	11 4
Sal.	13	10	7 3	0 2	2 4	5 5	5 5	4 1
San.		15 13	10 10	8 5	5 11	5 5	7 10	28
Spe.	11	15	20 20	12 8	7 7	9 15	9 7	10 10
Ste.		15	25	0 6	4 3	5 5	4 5	3 3
Tag.		19	26	14 13	17 9	13 12	10 10	10 10
Vol.	30	18	22 17	7 12	13 6	74 75	07 63	44 46
Wel.	10	15	15	0 7	15 2	7 6	6 8	6 8

These good conditions have not sufficed in past years to keep The Pelham Home free of streptococcal respiratory infections and rheumatic activity, however, they might have had some bearing on the results reported. A fourth study was therefore designed to test the possible protective action of sulfanilamide under conditions less favorable for the general well being of the subjects

This group consisted of 30 highly susceptible rheumatic subjects from 8 to 14 years of age. All were known to have contracted frequent streptococcal respiratory infections during previ-

*Most of them gave a strong familial rheumatic history. All had rheumatic heart disease with at least minimal involvement. Each had had one or more frank attacks of fever and hospital care. A few had 3 or 4 years study

ous years of observation. They were mostly from families with many children, living in the overcrowded rooms necessitated by their poor economic status. There was every reason to expect that many, if not all the members of the group, would contract at least one hemolytic streptococcal infection during the winter and spring months.

Procedure Most of these children were seen about 5 times a week, and all were examined by one of us twice a month. Each child received between 2 and 3 grams of sulfanilamide daily in three doses, spaced as evenly as possible, from November 1, 1937 to July 1, 1938. Clinical examinations, throat cultures, sedimentation rates, and blood sulfanilamide determinations were made twice a month. In addition, throat cultures were obtained from parents or siblings who reported respiratory infections.

Results Twenty of the 30 patients who started the study continued medication throughout the entire course. They will be referred to as Group A. The other ten (Group B) took the medication for only a part of the whole period, and served as controls.

In Group A none of the patients developed

drug manifestations. In Group B, the appearance of drug symptoms in 3 patients made it necessary for them to discontinue sulfanilamide during the first week of the experiment.

The concentration of sulfanilamide in the blood was maintained at an average level of from 31 to 62 gammas per cc, with a general average of 41 gammas for the whole group, as shown in Table V.

Five members of Group B stopped medication during the early fall because of drug symptoms. Three of these became infected with hemolytic streptococcus during the winter.

The other five remained under medication for 4 to 7 months, during which time none showed a positive throat culture. Of 4 who remained in New York City after stopping the drug, 3 contracted hemolytic streptococcal infection within 2 months. Altogether, 6 patients in Group B became infected after stopping sulfanilamide, and 3 of these infections were followed by rheumatic recrudescences. The occurrence of hemolytic streptococcus in the throat cultures of these 30 children are presented in Table VI.

TABLE V
The concentration of sulfanilamide expressed in gammas per cc in the blood of rheumatic children kept on maintenance medication in New York City between November 1937 and June 1938

Patient	Weight	Dose	Nov	Dec	Jan.	Feb	Mar	Apr	May	June	Average
	lbs	grams									
GROUP A											
Acc.	109	2 3	12	33	57 60	40 50	57 45	50 50	60 90	60	56
Ali	88	2 0	16	24	5 40	55 37	32 35	45 45	35 40	50	38
Alv	95	2 3	8	15	30 40	38 35	40 50	50 45	40 55	15	40
Bas	69	2 0	0 38	55	60 60	70 28	70 60	80 55	80 75	60	62
Ben	93	2 3	8	14	40 60	30 40	35 50	40 45	30	30	40
Cro	85	2 3	16	16	45 55	40 45	57 60	55 55	60 60		53
Cur	73	2 0		36	30 30	40 32	25 40	40 20	45 20	20	31
Fri	80	2 3	10	12	45 43	35 40	45 35	60 50	45 40	40	43
Hyl	132	2 3	8	20	30 30	30 18	6 35	35 25	17	30	26
Kir	101	2 3	8	22	35 40	33 30	30 45	40 30	50 50	40	38
Lui	99	2 3	6	12	40 40	35 40	33 35	45 35	40 40	35	39
Man	82	2 0	20	50	30 40	50 37	25 33	40 35	25 20	35	34
Mar	87	2 3	10	28	35 40	33 35	35 50	45 35	50 50	45	42
Mel	98	2 3	22	30	40 47	40 50	50 40	40 30	50 50	50	44
Pan	79	2 0	6	0	45 40	38 35	40 40	45 40	40 45	40	41
Ric	122	2 3		12	40 30	30 30	37 35	35 25	20 45	20	32
E Ry	98	2 0	8	0 35	10 33	33	35 50	45 30	30 20	55	34
F Ry	90	2 3	10	24 45	35 40	38 40	55 55	40 55	55 55	40	46
Tem	98	2 0	12	35 30	38 50	30 30	35 40	40 40	30 50	30	38
Van	104	2 3	4	28	14 32	45 30	35 33	35 30	40 25	40	33
GROUP B											
Bel	48	1 6			30 50	38	25 35	33 5	0		27
Ben	103	2 3	12 8	33	35 35	45 32	30 35	30 35	45 45	35	37
D'Or	64	2 0	12	30	55 55	60 50	55 55	70 70			59
Whe.	53	2 0	4	26	57	55	55 60				57
Zip	107	2 3			15 35	30 20	35 30	40 0			26

General average

It is not possible to estimate how frequently these children were exposed to infection in their normal home and school environment. However, it was determined that epidemics of streptococcal infection occurred in the families of 3 patients in Group A and did not affect the children taking sulfanilamide.

Only one patient (Cro) had hemolytic streptococcus in the throat flora at the onset of the experiment. One patient (Cur) who was admitted to the group December 1, with cervical adenitis, and who was started on medication at once, yielded a positive culture of hemolytic streptococcus 3 days later. We could not be sure whether infection with this organism preceded or followed the administration of the drug. It was possible in 3 other patients to obtain a few colonies of hemolytic streptococcus from the throat flora on one or two occasions. None of these organisms had the mucoid or matt colony appearance of virulent strains, nor did they give rise to respiratory infections. Their cultures died in the course of 2 months' storage in blood broth before they could be classified in Lancefield's groups.

In summary, this group of 20 patients appeared to escape streptococcal respiratory infection although a number of them had "common colds" and one developed measles. They also escaped clinical evidence of rheumatic fever. Their freedom from rheumatic activity can best be visualized from the record of their sedimentation rates, given in Table VII.

DISCUSSION

The variability of hemolytic streptococcus makes it impossible to predict what the incidence of infection will be in any particular year. Past observations at The Pelham Home over a period of 10 years have shown wide annual fluctuations in the prevalence of hemolytic streptococcus. The incidence has been as low as 25 per cent and in one epidemic year as high as 75 per cent (6). Likewise the prevalence of hemolytic streptococcal infections among our patients in New York City has varied a great deal. The average yearly incidence of infections from 1928 to 1938 was between 30 and 40 per cent. A control group of 400 non-medicated rheumatic subjects in New York City during 1938 were found to

TABLE VII

The blood sedimentation rates of rheumatic children in New York City under maintenance medication with sulfanilamide

Patient	Oct	Nov	Dec.	Jan.	Feb.	Mar	Apr	May	June
GROUP A									
Acc		18 25	30	20 10	15 10	15 6	15 10	18 5	10
Alv	12	10	6	8 12	10 9	8 4	6 18	7 10	
Bas		16 8	10	15 17	18 20	12 10	8 10	10 15	6
Ben	24	20 20	10	25 20	18 25	18 15	32 15	15 12	30
Cro		10 5	7	14 10	8 12	5 8	12 4	4 7	10
Cur		10 15	14 15	20 8	20 7	15 6	10 10	15 15	14
Fri			4	5 10	4 30	8 4	6 3	4 8	5
Hyl		10 12	12	5 15	6 5	10 8	6 5	10 6	7
Klr		3 10	15	4 5	10 4	9 2	4 5	7	7
Lul		11 10	17	18 10	10 10	15 9	7 7	5 6	5
Man		10 10	25	22 7	28 20	12 14	12 8	8 8	10
Mar		20 4	5	10 3	10 5	4 3	4 4	10 4	4
Mel		10 6	7	7 17	12 6	10 8	7 4	4 8	8
Pan		5 8	10	11 23	15 5	6 9	10 6	4 8	10
Ric		5 9	5	5 6	25 10	4 6	7 5	20 4	10
E Ry		10	9	14 10	10 4	15 12	10 8	5 6	5
F Ry	30	20	10	5 18	7 8		10 10	9 9	
Ten		10 15	10	7 7	7 3	4 12	12 4	7 10	5
Van	20	20	8	15 8	12 8	10 17	10 10	20 14	
	10	10	14	5 20	7 9	14 14	10 8	12 8	8
GROUP B									
Bel	8			6 8	12	10 12	8 12	20	
Ben		9 15	25	22 5	10 5	20 35	10 14	15 14	
D Or	8	5	10	12 10	5 5	10 10	5 7		
Whe	5	5	4		7	3 6			
Zip				5 22	8 18	6 2	4 4		
Bou		5							
Cam		8							
Ohr	5								
Wil		5 12	12						
Zel		5							

have an incidence of 35 per cent. Group A hemolytic streptococcal throat infections. The treated cases were selected because they had contracted pharyngitis frequently and experienced much rheumatic activity in the years preceding this study.

SUMMARY

Sulfanilamide administered to guinea pigs before or after the induction of streptococcal abscesses failed to sterilize the lesions.

Sulfanilamide used prophylactically prevented spontaneous infections and either prevented or modified the development of induced hemolytic streptococcal cervical adenitis in the guinea pig.

Sulfanilamide administered to rheumatic subjects after the onset of streptococcal throat infections did not prevent rheumatic recrudescences.

The possible prophylactic use of sulfanilamide was tested in 80 rheumatic children. Seventy-nine escaped hemolytic streptococcal infection and signs of rheumatic activity.

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BIBLIOGRAPHY

- 1 Massell, B. F., *Studies on the use of prontosil in rheumatic fever* New England J. Med., 1937 216, 487
- 2 Swift, H. F., Moen, J. K., and Hirst, G. K., *The action of sulfanilamide in rheumatic fever* J. A. M. A., 1938 110 426
- 3 Smith Theobald, *Spontaneous and induced streptococcus disease in guinea pigs. An epidemiologic study* Internat. Clin. 1931 3, 276.
- 4 Seastone, C. B., *The effect of sulfanilamide (para aminobenzenesulfonamide) on Group C⁺ hemolytic streptococcus infections.* J. Immunol., 1937 33 403
- 5 Lockwood, J. S., Coburn, A. F., and Stokinger H. E., *Studies on the mechanism of the action of sulfanilamide. The bearing of the character of the lesion on the effectiveness of the drug* J. A. M. A., 1938 111 2259
- 6 Coburn, A. F., and Pauli R. H., *Studies on the immune response of the rheumatic subject and its relationship to activity of the rheumatic process. II. Observations on an epidemic of influenza followed by hemolytic streptococcus infections in a rheumatic colony* J. Exper. Med., 1935 62, 137

STUDIES ON THE PHYSIOLOGY OF RESPIRATION IN PREGNANCY EFFECTS OF BARBITURATES ADMINISTERED DURING LABOR¹

By CHARLES P. SHELDON

(From the Department of Obstetrics, Harvard Medical School and the Boston Lying-in Hospital, Boston)

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It is becoming increasingly evident that there are certain dangers associated with obstetrical analgesia. Montgomery (1) investigated maternal mortality in Philadelphia and concluded that pentobarbital sodium was a significant factor. Gruber (2) emphasizes the dangers associated with anesthetic doses of the barbiturates, *i. e.*, depression of the cardiorespiratory mechanism and diminution of tonus in smooth muscle (bladder, ureter and uterus). Acute pulmonary edema may result from toxic doses or even therapeutic doses in individuals with an idiosyncrasy. Henderson (3) considers all narcotics and hypnotics used to produce obstetrical analgesia respiratory depressants affecting the baby more than the mother. The barbiturates are particularly dangerous because of the diminution of sensitivity to the normal respiratory stimulus—carbon dioxide. He believes they can be administered safely only in doses large enough to relieve anxiety.

This study was initiated as an attempt to determine the effects of the barbiturates on the respiratory mechanism of the normal parturient woman. It includes the following lines of investigation: (1) lung volume, (2) x-ray mensuration of diaphragm levels, (3) external measurements of the chest, and (4) intra-abdominal pressure.

LUNG VOLUME

Lung volume is an indicator of the functional status of the cardiorespiratory mechanism. Vital capacity has been utilized as an index of lung volume and variable results have been obtained. Alward (4) found a gradual reduction of vital capacity in the last month of pregnancy, a sharp reduction following delivery, and a gradual return to normal limits by the tenth postpartum day. Landt and Benjamin (5) found no significant

trend during the course of pregnancy although there was a monthly variation of 100 to 300 cc. from the mean. This was considered to be within normal limits. Thomson and Cohen (6) report a slight but usually progressive increase before delivery which could be correlated with a widening of the subcostal angle and was thought to be a direct result of mechanical alterations of thoracic volume. Vital capacity represents only one portion of the total lung volume and does not necessarily reflect similar changes in the other components (Figure 1). Christie (7) has devised a technique whereby all of the components of lung volume can be determined. A presentation of the following material will be simplified by definitions taken from Christie's paper.

Vital capacity is the volume of air that can be forced out of the lungs by maximum expiration following the fullest inspiration.

Tidal air is the volume of air expired by a breath of average size.

Subtidal air is the volume of air remaining in the lungs after a normal expiration.

Residual air is the volume of air remaining in the lungs following maximum expiration.

Complemental air is the amount of air inspired from the height of a normal inspiration to maximum inflation.

Reserve air is the volume of air expired from a normal expiration to maximum deflation.

Total capacity is the sum of vital capacity and residual air.

PROCEDURE

Complete lung volume studies were made on seventeen patients who were delivered on the obstetrical service of the Albany Hospital. The determination of the subtidal air using Christie's technique, was made with the patient in a supine position, the arms resting at the sides and the head supported by one pillow. Care was taken to prevent heavy bed clothing from resting upon the abdomen or chest. Binders were unpinned. Vital capacity studies were then performed with the patient in the same carefully controlled position. The complementary and supplementary (reserve air) volumes were computed from the vital capacity. Residual air was determined by sub-

¹This study was carried out under Grant 424 of the Committee on Scientific Research of the American Medical Association.

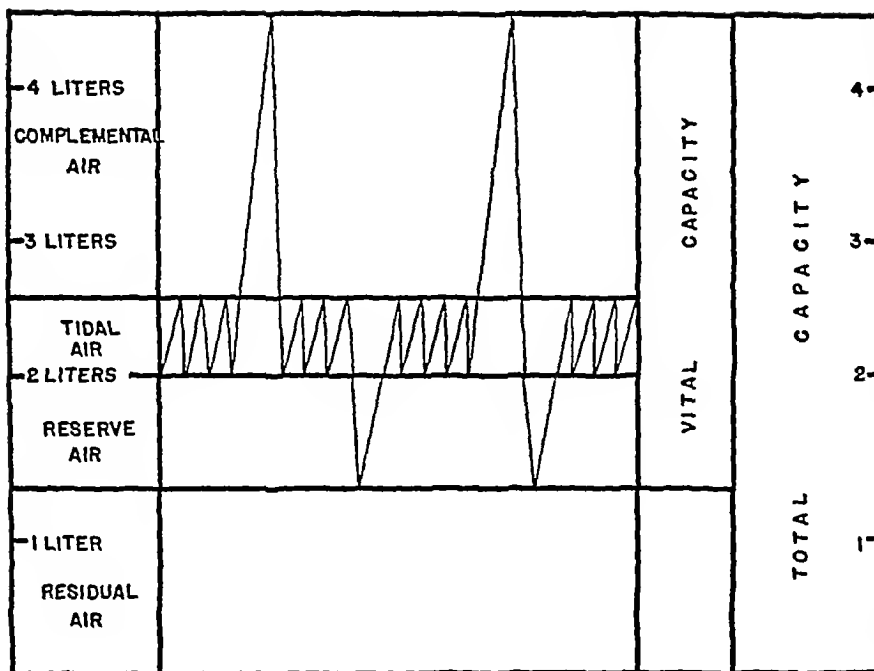


FIG 1 LUNG VOLUME AND ITS SUBDIVISIONS

Total lung volume and its subdivisions are expressed in liters Absolute values are taken from Hurtado *et al* (15)

tracting the reserve air from the subtidal air Patients were examined under basal conditions insofar as possible. Determinations were made before delivery and at varying stages of the puerperium. The first three days after delivery were investigated more intensely than other stages of the postpartum period because maximum changes occur soon after delivery Results were expressed in terms of percentage of normal lung volume which was arbitrarily taken, because of Alward's findings, as that found on the tenth postpartum day Duplicate analyses were always obtained. All volumes were corrected to 37° C. saturated with water vapor

Sixteen patients of the series investigated were delivered through the pelvis This group can be divided into the following sub-groups (a) four patients were delivered without analgesia or anesthesia, (b) eight patients were given one of the barbiturates and scopolamine during labor and ether anesthesia at delivery, (c) two patients were given one of the barbiturates and scopolamine during labor but no anesthesia at delivery, and (d) two patients had no analgesic drugs during labor but were given ether anesthesia at delivery The barbiturates administered during labor were seconal, sodium amytal, and pentobarbital sodium. Twelve grains of sodium amytal was the largest dosage used. There was a single patient delivered by cesarean section. Atropine grains 1/150 was given preoperatively and gas-oxygen-ether was used as the anesthetic.

RESULTS

The total lung volume shows no significant change although the vital capacity tends to increase slightly in the latter part of pregnancy The average value for all vital capacity determinations made during the last month of pregnancy was seven per cent above normal in this series (Figure 2)

Patients delivered without analgesia or anesthesia showed either no significant changes or an increase of the total lung volume and its components during the first week following delivery

Barbiturate and scopolamine analgesia during labor plus ether anesthesia at delivery results in an eight to ten per cent reduction of total volume, functional residual air (subtidal air) and vital capacity as late as the fourth and fifth days of the puerperium The reduction of vital capacity is actually fifteen to twenty per cent below the antepartum level The reduction of lung volume is even more striking in the first twenty-four hours after delivery when depression is maximum

Barbiturates alone decreased the total volume

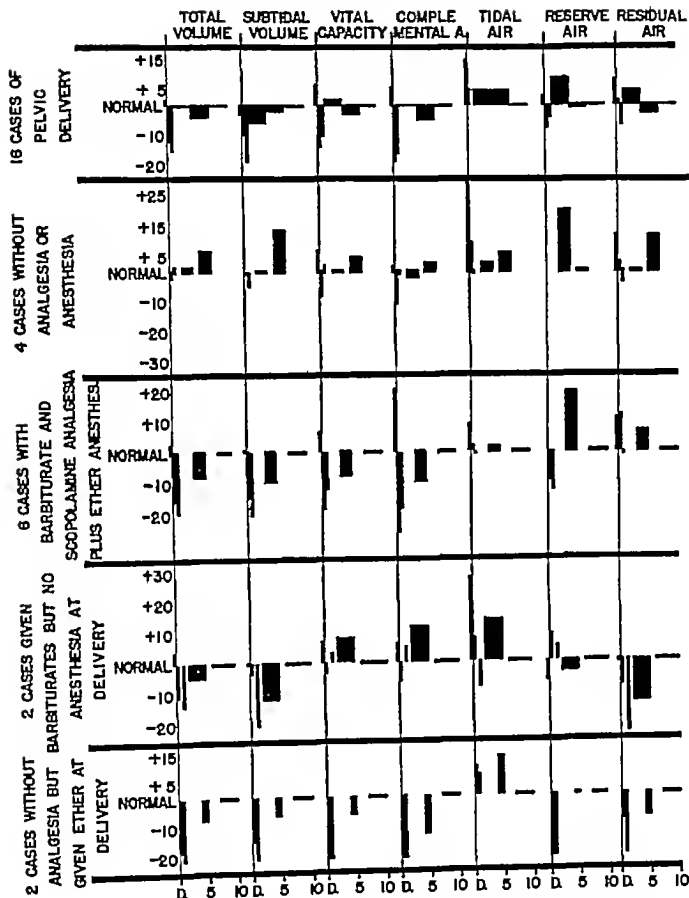


FIG. 2. THE EFFECT OF OBSTETRICAL ANALGESIA ON LUNG VOLUME

Vertical lines designated as "D" represent delivery of the patient. The total lung volume and its components have been expressed in terms of percentage of normal which was taken arbitrarily as that value obtained on the tenth postpartum day

and the subtidal air but the vital capacity remained unaffected

Ether anesthesia alone decreased the total volume, subtidal air, and vital capacity but the reduction was less than that brought about by a combination of analgesia and anesthesia.

The patient delivered by cesarean section showed a marked reduction of vital capacity following delivery with a proportionately smaller reduction of total volume. These results indicate the effects of postoperative pain and posture associated with laparotomy.

Cesarean section reduced the vital capacity markedly. On the other hand, the functional residual air (subtidal volume) increased. An increase of the residual air, which is one of the components of the subtidal volume, compensated for the reduction of vital capacity. The total lung volume therefore was not diminished as much as the vital capacity (Table I).

TABLE I
The effect of cesarean section on lung volume and its components

Stage	Total volume	Subtidal volume	Vital capacity	Complete mental air	Tidal air	Supplemental air	Residual air
Antepartum	cc	cc	cc	cc	cc	cc	cc
Postpartum 7 hours	3426	2537	974	444	445	85	2452
Postpartum 3 days	4706	2915	1949	1429	372	175	2740
Postpartum 6 days	4211	2471	1973	1432	308	233	2238
Postpartum 12 days			2191	1626	347	217	
Postpartum 20 days	4404	2156	2659	1967	281	411	1745
Postpartum 7 weeks	5015	2486	2897	2226	303	368	2118

External measurements of the chest

Since the lungs are encased in a bony cage—the thorax—some light may be thrown on the physiology of respiration during pregnancy and the puerperium by measurements of the size of the chest (Figure 3). The external measurements have been expressed in terms of percentage

of normal. The values for circumference and diameter found on the tenth postpartum day were taken as normals. Measurements were made at the level of the submammary crease.

The anteroposterior diameter of the chest was fifteen to twenty per cent above normal during the last two weeks of pregnancy. The circumference was increased approximately ten per cent. We have seen that total lung volume remains essentially unchanged during pregnancy in spite of an apparent increase in the size of the thoracic cavity. Flaring of the ribs may therefore be a mechanical factor which compensates for a cephalic displacement of the diaphragm by the advancing pregnancy.

X-ray mensuration of diaphragm levels

Landt and Benjamin (5) have observed elevation of the diaphragm from the fourth month on. By the middle of pregnancy the elevation averaged two centimeters above normal at the end of deep inspiration. McGintry (8) found higher diaphragm levels and a greater excursion antepartum than postpartum. Lewis (9) has demonstrated experimentally the importance of activity of the diaphragm in maintaining the normal relationship between intra-abdominal and intrathoracic pressures.

Immediately following delivery a descent of the diaphragm occurs which is of great importance as this increases intra-abdominal pressure and restores a normal balance. Case 1 delivered without analgesia or anesthesia demonstrated a

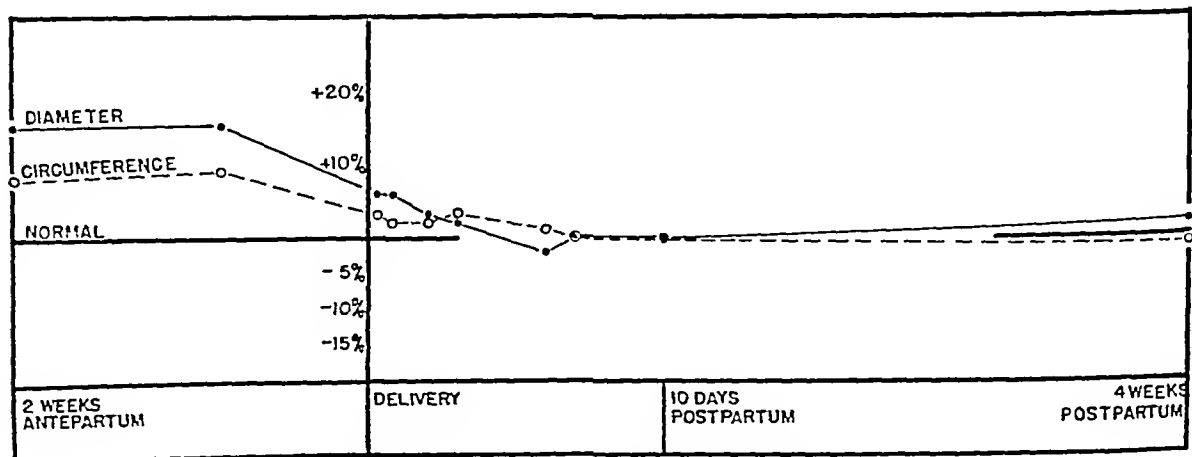


FIG 3 EXTERNAL MEASUREMENTS OF THE CHEST

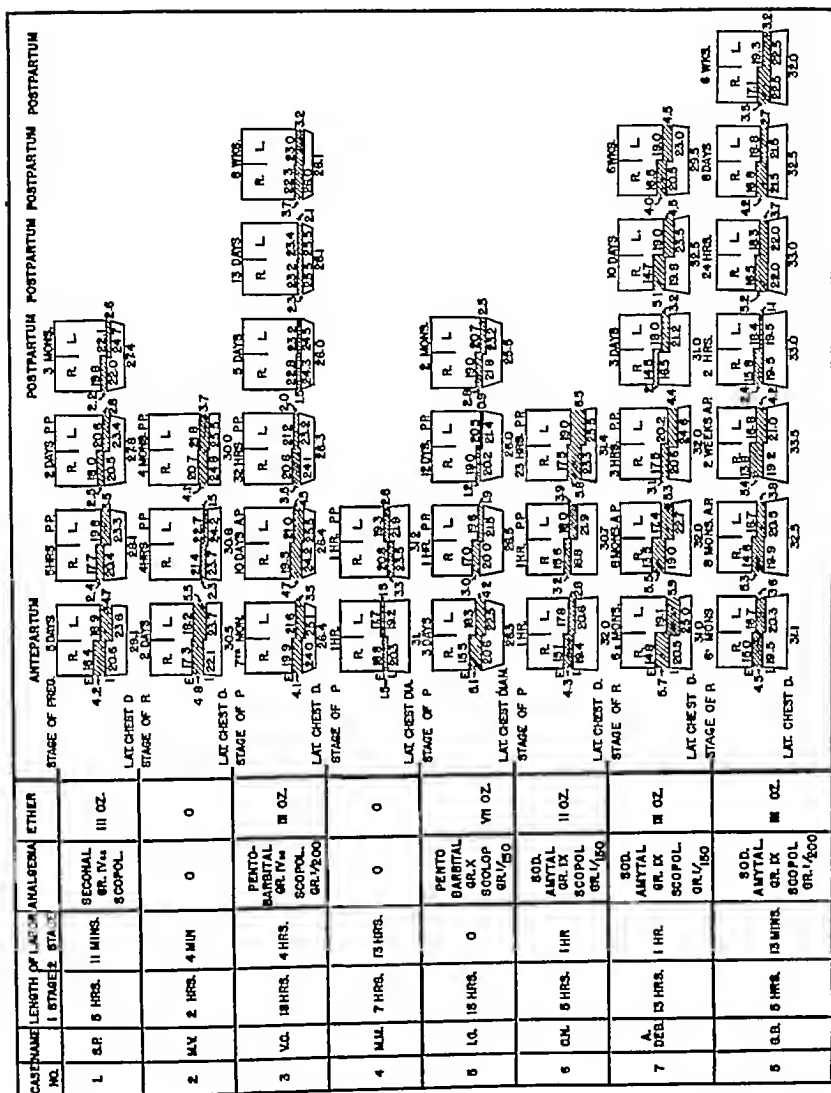


FIG. 4 ALTERATIONS IN DIAPHRAGM LEVELS DURING PREGNANCY AND THE PUERPERIUM

descent of the diaphragm measuring 16 cm four hours after delivery. Case 4 similarly delivered showed a descent of 32 cm one hour after delivery. Descent of the diaphragm was retarded by the depression associated with analgesic drugs during labor and anesthesia at delivery.

Figure 4 illustrates the alterations in diaphragm levels during pregnancy and the puerperium. Roentgenograms were taken at the end of maximum inflation and maximum deflation. The first three cases were x-rayed at 84 inches with the patient always in a sitting position. The remainder were x-rayed in a supine position with the distance from target of the Coolidge tube to the plate kept constant at 30 inches. Measurements were made from the first rib to the dome of the diaphragm and are expressed in centimeters. "E" represents expiration and "I" inspiration. The cross-hatched area represents the excursion of the diaphragm.

Intra-abdominal pressure

Coombs (10) considers the intra-abdominal pressure lower during pregnancy than under normal conditions in spite of an increase in the contents of the abdominal cavity. Schatz (11) suggests that there is an adjustment of the abdominal muscles to compensate for distention of the pregnant uterus. Emerson (12) concludes from animal experimentation that normally intra-abdominal pressure is above atmospheric pressure and fluctuates with the respiratory excursion of the diaphragm and thorax. There is a decrease of intra-abdominal pressure whenever the tone of the diaphragm and abdominal muscles is diminished. Following delivery we have observed a diminished respiratory excursion in spite of the fact that the diaphragm has less resistance to contract against. Due to failure of the abdominal recoil expiration is only partially accomplished.

A bag measuring one and one-half inches in diameter by three inches in length was made of the same non-distensible material as is used in Voorhees' bags. This was attached to a mercury manometer by means of a rigid rubber tube which would transmit pressure changes only in its long axis. The bag was distended with water by means of a burette attached to the upright arm of the manometer. The system was adjusted so

that the mercury levels in the U-tube were equal when the bag was at the level of the rectum. The bag was then deflated, inserted into the large bowel and reinflated with an equal quantity of water. Pressures were expressed in millimeters of mercury.

Intrarectal pressure readings were performed on five patients in an attempt to ascertain indirectly the changes in intra-abdominal pressure which follow delivery (Table II). There was

TABLE II
Intrarectal pressure readings

Patient	Stage	Intra- rectal pressure	Reduction
		mm. Hg	per cent
Mrs. L.	Antepartum	24	50
	1 hour postpartum	12	
	1 month postpartum	36	
Mrs. DeM.	Antepartum	22	77
	1 hour postpartum	17	
Mrs. G.	Antepartum	17	41
	1 hour postpartum	7	
	10 days postpartum	18	
Mrs. F. C.	Antepartum	26	77
	1 hour postpartum	20	
Mrs. M. M.	Antepartum	28	61
	3 hours postpartum	17	

roughly a fifty per cent reduction in intrarectal pressure immediately following delivery. Weisker (13) considers the pressure in hollow viscera to be independent of intra-abdominal pressure because wide variations occur in the contents of the cavity and elasticity of its wall. Nevertheless, it seems reasonable that intra-abdominal pressure should decrease following delivery because of the sudden alteration in the size of the uterus after expulsion of the products of conception thereby decreasing the contents of the abdominal cavity.

DISCUSSION

Vital capacity is considered a fair index of cardiac and respiratory function. In attempting to measure this component of the total lung volume during pregnancy and the puerperium it is necessary to take into consideration all extraneous factors which may have an effect such as fatigue, posture, sedation, etc. A patient who is given

no analgesia or anesthesia should not be compared with patients depressed by such measures. From experimental evidence it seems reasonable to assume that after the exclusion of the reduction that results from fatigue of a long labor, which may be negligible, there is no significant change in the vital capacity following pelvic delivery provided labor is conducted without analgesia and delivery is carried out without anesthesia. If, on the other hand, barbiturates and scopolamine are administered during labor and ether is given at delivery, there is a significant reduction of the vital capacity below the antepartum level. If we take into account an abdominal operation such as cesarean section we observe a rather marked reduction of vital capacity but the decrease of total lung volume is proportionately much less.

There would seem to be a mechanism which prevents a serious reduction of the functional residual air (subtidal volume) following delivery, whether pelvic or abdominal in type. A reduction of intra-abdominal pressure after delivery and a subsequent descent of the diaphragm are undoubtedly significant factors. The diaphragm is the most important muscle of respiration since it is the direct expander of the lower lobes. Its position and excursion are closely linked with the relationship between intra-abdominal and intra-thoracic pressures. Prinzmetal and Kountz (14) record a moderate rise of the intrapleural pressure late in pregnancy owing to ascent of the diaphragm. Following delivery, the decrease of intra-abdominal pressure is compensated for by descent of the diaphragm which results in a rise of pressure during inspiration. If diaphragmatic activity and descent are diminished by loss of tonus caused by excessive dosage of drugs during labor, this mechanism is disturbed and the intra-abdominal pressure will approach the intrapleural pressure. Efficient respiration depends upon the pressure in the pleural cavity being much more negative than that in the peritoneal cavity. Without this pressure difference elevation of the diaphragm during expiration will be greatly limited. The retardation in descent of the diaphragm in patients given barbiturates during labor permits deflation of the lungs, accumulation of mucus and closure of bronchi. Absorption of air from the occluded areas results in atelectasis. Tonus of the diaphragm and thoracic muscles

keeps the lungs and thorax expanded and prevents atelectasis.

CONCLUSIONS

- 1 Lung volume determinations, using Christie's technique, were performed on a limited number of puerperal patients. Determinations were made at varying stages of pregnancy and the puerperium.

- 2 The total lung volume shows no significant alterations as a result of pregnancy.

- 3 The vital capacity normally increases slightly in the antepartum period. If labor is conducted without analgesia and delivery is performed without anesthesia there is a moderate reduction in this component of the total lung volume following delivery which rapidly returns to normal.

- 4 Respiratory depression resulting from large doses of analgesic drugs during labor or profound anesthesia at delivery will alter the cardiorespiratory mechanism so that the vital capacity is markedly reduced in the postpartum period and its return to normal is delayed.

- 5 Cesarean section is accompanied by a marked reduction of vital capacity and a proportionately smaller reduction of total volume.

- 6 Alterations of thoracic volume from flaring of the costal margins compensates for a cephalic displacement of the diaphragm by the advancing pregnancy. The total lung volume is thereby maintained at approximately its normal level.

- 7 An increased excursion of the diaphragm as pregnancy advances assists in maintaining normal aeration of the lungs and compensates for any possible atelectasis of the lung bases as a result of compression by the encroaching diaphragm.

- 8 Descent of the diaphragm following delivery results in a rise of intra-abdominal pressure during inspiration thereby assisting the respiratory mechanism which otherwise would be embarrassed by the reduction of expiration owing to failure of the abdominal recoil. Experimental evidence suggests that diaphragmatic activity and descent are diminished by loss of tonus resulting from excessive dosage of drugs during labor.

- 9 Barbiturates are respiratory depressants and should not be used in doses that will produce more than a sedative effect during labor.

BIBLIOGRAPHY

- 1 Montgomery, T L, Obstetric amnesia, analgesia and anesthesia, their relationship to sudden death in labor J A M A., 1937 108, 1679
- 2 Gruber, C. M, Certain pharmacologic actions of the newer barbituric acid compounds Am J Obst. and Gynec., 1937, 33, 729
- 3 Henderson, Y, The Pharmacopeia and the physician, respiratory stimulants and their uses J A M A., 1937, 108, 471
- 4 Alward, H C., Observations on vital capacity during last month of pregnancy and puerperium. Am J Obst. and Gynec., 1930, 20, 373
- 5 Landt, H, and Benjamin, J E, Cardiodynamic and electrocardiographic changes in normal pregnancy Am. Heart J, 1936, 12, 592.
- 6 Thomson, K. J., and Cohen, M E., Vital capacity observations in normal pregnant women. Surg Gynec., and Obst., 1938 66, 591
- 7 Christie, R. V, Lung volume and its subdivisions, methods of measurement J Clin Invest., 1932, 11, 1099
- 8 McGintry, A P, The comparative effects of pregnancy and phrenic nerve interruption on the diaphragm and their relation to pulmonary tuberculosis Am J Obst and Gynec., 1938, 35, 237
- 9 Lewis, T, Studies on the relationship between respiration and blood pressure. J Physiol, 1908, 37, 213
- 10 Coombs, H C, Mechanism of the regulation of intra-abdominal pressure Am. J Physiol, 1922, 61, 159
- 11 Schatz Quoted by Emerson (12)
- 12 Emerson, H, Intra-abdominal pressure. Arch Int. Med, 1911, 7, 754
- 13 Weisker Quoted by Emerson (12)
- 14 Prinzmetal, M, and Kountz, W B, Intrapleural pressure in health and disease and its influence on body function Medicine, 1935, 14, 457
- 15 Hurtado, A, Fray, W W, Kaltreider, N L., and Brooks, W D W, Studies of total pulmonary capacity and its subdivisions, normal values in female subjects J Clin. Invest., 1934 13, 169

A COMPARISON OF THE EFFECTS OF VITAMIN D DIHYDROTACHYSTEROL (A.T. 10), AND PARATHYROID EXTRACT ON THE DISORDERED METABOLISM OF RICKETS

By FULLER ALBRIGHT HIRSH W. SULKOWITZ AND ESTHER BLOOMBERG

(From the Medical Service of the Massachusetts General Hospital and the Department of Medicine of the Harvard University Medical School Boston)

(Received for publication September 16 1938)

In a previous paper (1) it was concluded that vitamin D has two fundamental and separate actions (a) to increase the absorption of calcium from the gastro intestinal tract, and (b) to increase the phosphate excretion in the urine. In a later paper (2) evidence was brought forth which suggested that A.T. 10 (dihydrotachysterol) has both these properties but that the ratio of the calcium absorption factor to the phosphate excretion factor is less with A.T. 10 than with vitamin D. It was pointed out (2), furthermore, that the effect of either A.T. 10 or vitamin D on phosphate excretion may be masked in patients with their parathyroids intact by a tendency in the opposite direction resulting from decreased parathyroid activity occasioned by the increased calcium absorption. It was thought that the reason A.T. 10 was not antirachitic was because of this increased effect on phosphate excretion, a property obviously not antirachitic. Finally it was pointed out (2) that the parathyroid hormone differed in action from both vitamin D and A.T. 10 in having only the effect on phosphate excretion in the urine without any effect on calcium absorption.

It was decided to test the above conclusions by studies on a patient with chronic infantile rickets. The subject of the investigation was a boy of 17 with vitamin D resistant rickets. He was the same boy who was previously reported (3) on whom it was shown that the disordered metabolism was essentially the same as in the usual form of rickets but to whom it was necessary to give massive doses of vitamin D before therapeutic results were obtained. In the present investigation the metabolic effects of A.T. 10 and parathyroid extract were studied. These data can be compared with those from similar studies already reported with vitamin D on the same patients (3). Whereas these latter studies were conducted approximately 5 years before the un-

derlying metabolic disorder was essentially the same during both studies. To be sure, the bones were much less rachitic during the present investigation. On the other hand the blood values—serum calcium phosphorus and phosphatase—were almost identical at the beginning of both investigations.

METHODS

Vitamin D therapy was discontinued 78 days before the present investigation was commenced. The patient received a weighed low calcium, moderately high phosphorus diet of similar composition throughout the 16 3-day metabolic periods. Five grams of calcium lactate were added to the diet daily. The patient was on this régime for 4 days before collections were started. It should be noted that it is much better when one wants a constant high calcium diet to give a low calcium diet and add a pure calcium salt. The inevitable slight fluctuation in the composition of the food thus becomes negligible.

DISCUSSION OF RESULTS

The data are shown in Table I and Figure 1. During the three control periods (I, II, III), the serum calcium was almost normal (9.9 and 9.3 mgm. per 100 cc.), the serum phosphorus was very low (1.7 and 1.9 mgm. per 100 cc.), there was a large amount of calcium and phosphorus in the feces, the calcium metabolism was practically in balance, and there was a negative phosphorus balance partly attributable to a loss in weight.

During Periods IV, V, VI, and VII the patient received 25 mgm. of A.T. 10 twice daily. The fecal calcium excretion was markedly decreased going from 1.60 grams in Period III to 0.20 gram in Period VIII, the first period after cessation of therapy. The blood serum calcium rose to a high level of 13.3 mgm. per 100 cc. on

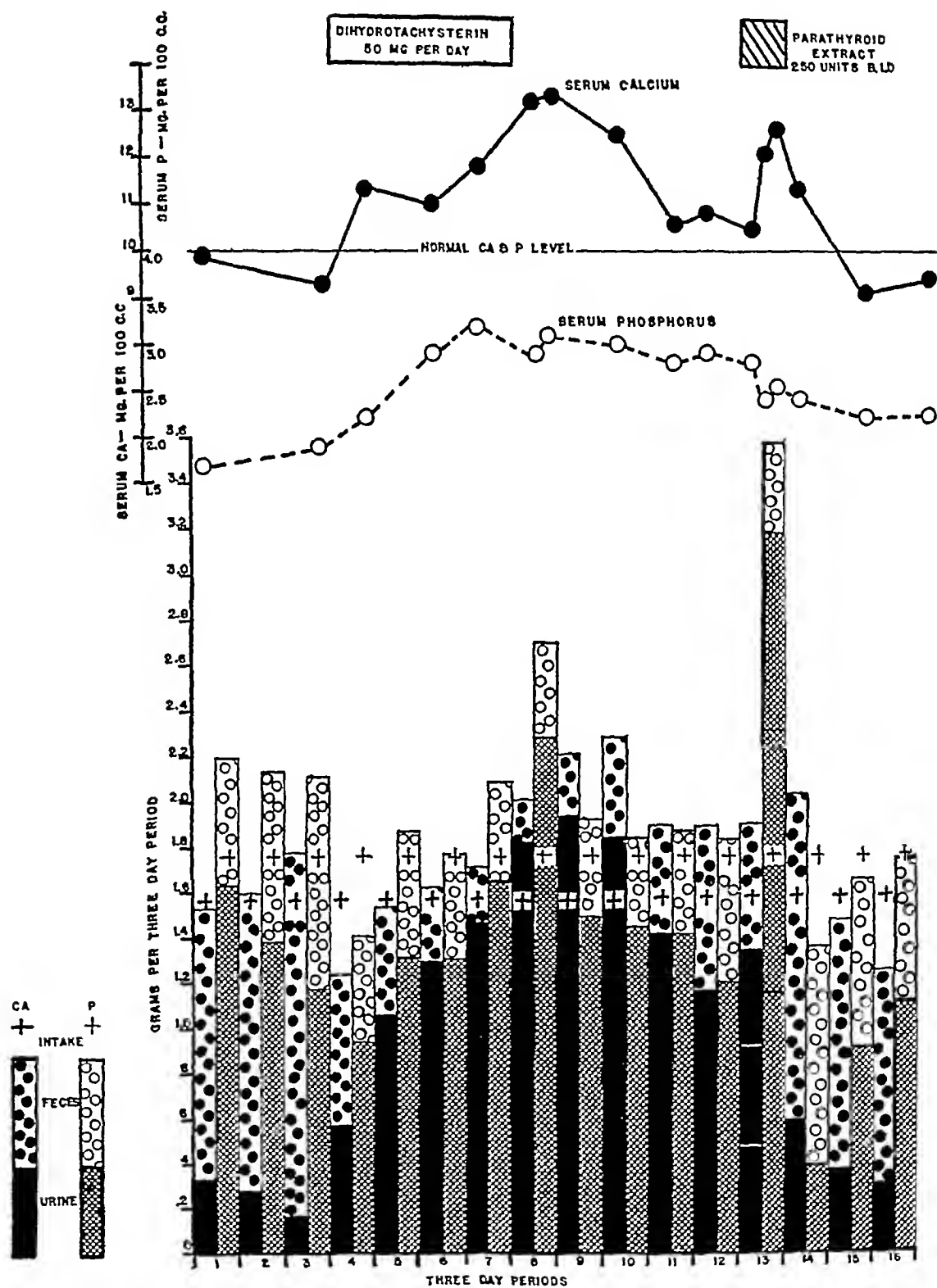


FIG 1 A GRAPHIC PRESENTATION OF DATA IN TABLE I SHOWING EFFECTS OF AT 10 AND OF PARATHYROID EXTRACT ON DISORDERED CALCIUM AND PHOSPHORUS METABOLISM OF INFANTILE RICKETS

Division marks in columns representing urinary excretions for Period 13 are to indicate amount of excretion on each of the three days

TABLE I
Metabolic data showing effect of A T 10 and of parathyroid extract

Pe- riod	Date	Weight of patient	Calcium				Phosphorus				Serum			Treatment
			Urine	Feces	In take	Bal- ance	Urine	Feces	In take	Bal- ance	Cal- cium	Phos- phorus	Phos- phatase	
		kgm.	grams	grams	grams	grams	grams	grams	grams	mgm. per 100 cc.	mgm. per 100 cc.	Bodansky units		
I	Dec. 1937													
II	12 13-14	46.5	0.33	1.19	1.56	+0.04	1.63	0.57	1.76	-0.44	9.9 I*	17		
III	15-16-17	46.2	0.28	1.32	1.56	-0.04	1.38	0.75	1.76	-0.37				
IV	18-19-20	45.9	0.16	1.60	1.56	-0.20	1.17	0.93	1.76	-0.34	9.3 III	1.9	7	
V	21 22 23	45.8	0.37†	0.65	1.56	+0.30	0.94†	0.46	1.76	+0.36	11.3 III	2.3	10	
VI	24-25-26	46.0	1.06	0.47	1.56	+0.03	1.51	0.56	1.76	-0.11				
VII	27 28-29	45.8	1.29	0.34	1.56	-0.07	1.30	0.47	1.76	-0.31	11.0 I	2.9	9	
VIII	30-31 Jan. 1	45.9	1.46	0.25	1.56	-0.15	1.64	0.44	1.76	-0.32	11.2 I	3.2	8	
	2-3-4	45.8	1.81	0.20	1.56	-0.45	2.28	0.42	1.76	-0.94	13.2 II	2.9	6	
										13.3 III	3.1	7		
IX	5-6-7	45.3	1.93	0.28	1.56	-0.65	1.48	0.44	1.76	-0.16				
X	8-9-10	45.2	1.84	0.45	1.56	-0.73	1.44	0.59	1.76	-0.07	12.5 I	3.0	9	
XI	11 12 13	45.2	1.40	0.40	1.56	-0.33	1.41	0.45	1.76	-0.10	10.6 II	2.8	8	
XII	14-15-16	45.3	1.16	0.73	1.56	-0.33	1.20	0.62	1.76	-0.06	10.8 I	2.9	11	
XIII	17	45.8	0.46				1.18				10.8	2.8	11	Parathyroid extract 500 U
	18	44.8	0.46				1.18				11.1	2.4	11	Parathyroid extract 500 U
	19	44.7	0.48				0.87				11.5	2.4	11	Parathyroid extract 500 U
	17-18-19		1.34	0.56	1.56	-0.34	3.18	0.38	1.76	-1.80				Parathyroid extract 1 500 U
XIV	20-21 22	44.7	0.59	1.44	1.56	-0.47	0.38	0.96	1.76	+0.42	11.3 I	2.4	10	
XV	23-24-25	45.3	0.37	1.10	1.56	+0.09	0.91	0.74	1.76	+0.11	9.1 III	2.2	13	
XVI	26-27 28	45.1	0.30	0.95	1.56	+0.31	1.10	0.64	1.76	+0.02	9.4 III	2.2	10	

* Roman numerals indicate to which day of period data pertain.

† One of three urine specimens lost during this period. Figures obtained by multiplying values for other two days by 3/2. This probably not justified as values were undoubtedly changing rapidly from day to day.

the third day of Period VIII. The urinary calcium excretion rose as much as and finally more than the fecal calcium excretion decreased so that the calcium balances instead of becoming positive eventually became negative.

The urinary phosphorus excretions are most instructive. Period IV had best be disregarded as the urine for one day was lost by accident and the values recorded were obtained by multiplying the excretions during the remaining two days by 3/2. It is quite clear that A.T. 10 (see Periods V to VIII) caused a definite increase in the urinary phosphorus excretion. The increase was not tremendous as occurs when A.T. 10 is given to an individual without parathyroid tissue (2). This may be explained by the hypothesis that the phosphorus-excretion property of A.T. 10 was masked by the decreased activity of the parathyroid glands secondary to the elevated serum calcium (*vide supra*). On the other hand, phosphorus excretion in the urine was increased whereas with vitamin D it had been decreased (Table II). This is in accordance with the hypothesis that A.T. 10 has a relatively greater effect on phosphorus excretion than vitamin D.

The fecal phosphorus excretion with A.T. 10 was decreased but not to the same extent as the

fecal calcium excretion. The serum phosphorus values rose (1.9 mgm to 3.1 mgm per 100 cc.) presumably because of decreased parathyroid activity resulting from increased calcium absorption. This rise of the serum phosphorus does not occur of course, when A.T. 10 is given to parathyroidless individuals (2). The phosphorus balances on the whole tended to become more negative. This fact becomes more definite if one compares the balances with the two control periods at the end of the experiment (Periods XV and XVI) as well as with Periods I, II and III when there was a negative phosphorus balance probably attributable to a negative nitrogen balance (*cf* weights in Table I).

During the remainder of the experiment, except for Periods XIII and XIV when there was a temporary disturbance resulting from the administration of parathyroid extract in Period XIII one can note the gradual return of the values to the pre A.T. 10 levels.

On each day of Period XIII the patient received 250 units of parathyroid extract twice daily. This resulted immediately in an almost threefold increase in the urinary phosphorus excretion and a large negative phosphorus balance. The moderate decreases in the fecal calcium and phos-

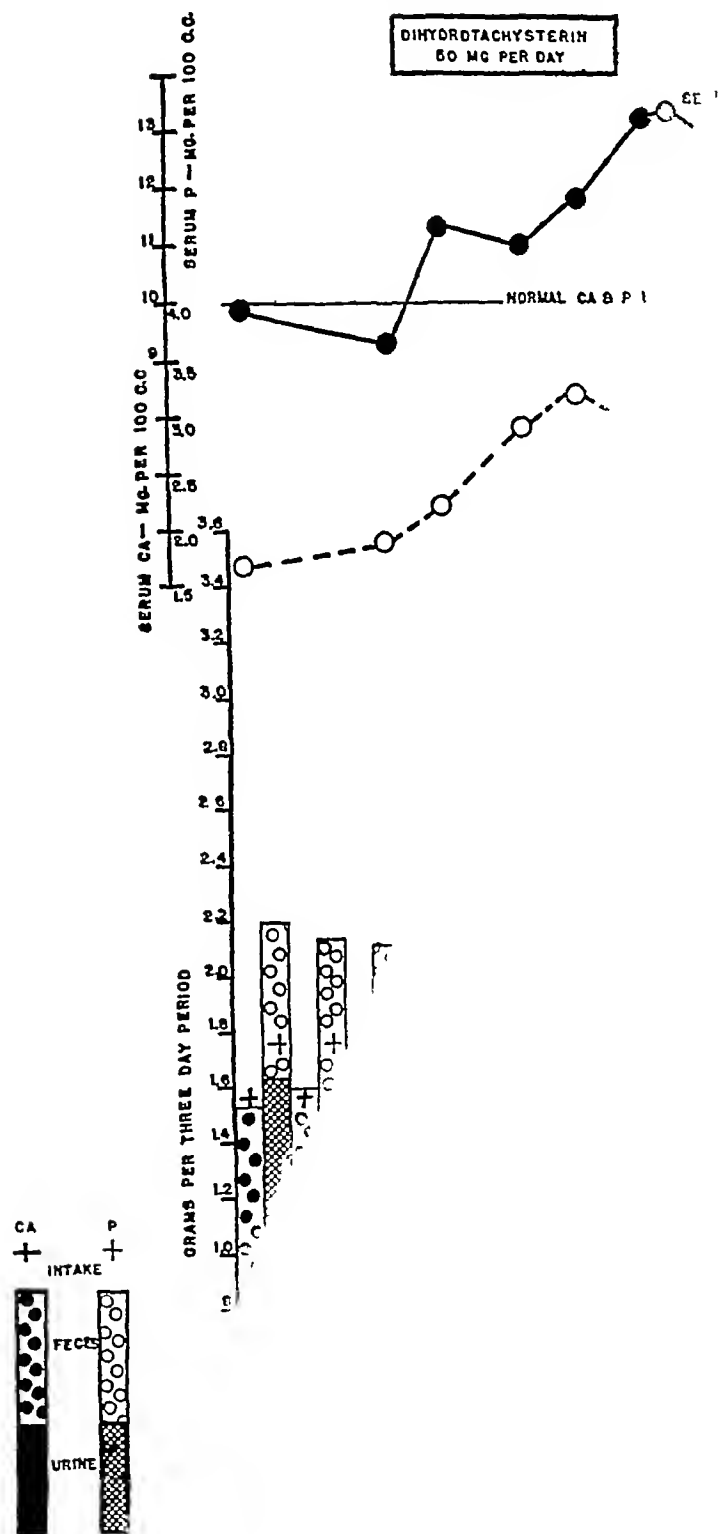


FIG 1 A

Divide
on each

GONADOTROPIC HORMONE URINE ASSAYS OF NORMALLY CYCLING, MENOPAUSAL, CASTRATED AND ESTRIN TREATED HUMAN FEMALES¹

By CARL G. HELLER AND EMILY J. HELLER

(From the Department of Medicine University of Wisconsin Medical School Madison)

(Received for publication October 11 1938)

Despite the numerous reports on the hormone content of human blood and urine, only a hazy concept of the actual and relative content of gonadotropic substance in urine or blood exists. As recently as June 10 1937 the consensus at the meeting of the Section on Obstetrics, Gynecology, and Abdominal Surgery at the Eighty-Eighth Annual Session of the American Medical Association seemed to be that, although the hormone assays were of scientific interest, they had little clinical value as yet. To quote two opinions concerning hormone assays, Emil Novak said, "The papers we have heard today illustrate the usual inadequacy of blood and urine hormone studies in pointing the way toward successful treatment" (1), and Elmer Sevringhaus said, "These studies are still to be reserved for the highly experimental clinics because assay techniques are far from being uniformly reliable." (2)

The three most important underlying difficulties with the gonadotropic assays to date appear to be (1), lack of adequate assay methods (2), lack of reliable concentration methods and (3) lack of a thorough knowledge of the normal physiology of gonadotropic hormones in the human being.

The assay methods have recently been improved by the introduction of reliable techniques using the immature mouse (3) and the immature rat (4). The problem of urine concentration has also been simplified recently by Levin and Tyndale (5) who introduced the tannic acid method, and by Heller and Heller (6) who have successfully applied Zondek's alcohol ether concentration method. This paper attempts to add information concerning the third problem namely the excretion rate

of the gonadotropic hormone of women during and after the menopause.

The specific questions we have tried to shed light on are

(a) Is there any relationship between age and gonadotropic hormone titer?

(b) Is there a constant relationship between gonadotropic excretion of menopausal women who have symptoms and of those who do not?

(c) Is the gonadotropic content of urine reduced after the menopause is passed?

(d) Do surgical or radiation castrates differ from menopausal women in their gonadotropic excretion, is the amount of time elapsed since castration important?

(e) What is the urinary gonadotropic excretion of patients with involutional psychoses?

(f) What is the urinary gonadotropic excretion of patients who have been hysterectomized?

(g) What is the urinary gonadotropic excretion of normally cycling women of menopausal age?

(h) How rapid and how great is the change from the cycling condition to the menopausal condition?

However, before these questions are answered it is necessary to present more specific information about the excretion of gonadotropic hormones by normal menopausal women. The following facts will be discussed, the extent of the normal daily variation in excretion, how dilution, concentration and time of voiding (that is, night or day specimens) vary the assays and whether women still exhibit cycles of excretion after the climacteric.

MATERIALS AND METHODS

In most instances, the patients were hospitalized cases in no instance was there any obvious metabolic disturbance other than the menopause syndrome. Urine collections were made on both a 12 hour (overnight) and a 24-hour

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basis The urine was placed in an ice chest immediately upon voiding, no preservatives were used. An aliquot sample of the total 12 or 24-hour specimen was precipitated. The final eluted material was so divided as to represent a specific fraction of the original volume, this fraction is referred to as the "equivalent dose." The concentration method has been previously described (6) The rats were slightly older than in previous reports (4, 6), 24-day-old rats being used because of their better tolerance for the concentrates Graded equivalents of the original urine were injected in 0.5 or 1.0 cc. doses 2 times per day for 3 days, autopsies were performed on the fourth day Since from 1 to 10 rats were injected with different equivalent doses, a theoretically complete curve of response to dose was determined for each specimen of each patient. From this curve the minimal volume of urine necessary to produce a minimal uterine stimulation was approximated. The ratio between doses causing minimal uterine weight changes and doses causing minimal ovarian weight changes was roughly one to two In most cases, minimal stimulation was roughly calculated from the observed response to known equivalents This is illustrated by a typical case (Table I) where 6 rats were used on 4 different dose

The conclusion in this case was that the minimal dose must lie between 25 cc. and 125 cc. ents, since the response was quite positive at 25 cc. and completely negative at 12.5 cc. A working unit of 18 was therefore adopted. It is recognized that this is a rough estimate, and that it could be improved by using more animals and more dose levels However, it

TABLE I

Illustrating the results of gonadotropic assays on a single urine specimen

NAME ST DATE April 22, 1938 24-HOUR COLLECTION
TOTAL VOLUME 1340 cc ALIQUOTS 500 cc.
METHODS OF PRECIPITATION Alcohol-ether
DATE OF PRECIPITATION April 24
DATE OF INJECTION April 26 AGE OF RATS 24 days

Dose*	Amount injected	Uterine weight + fluid	Uterine weight - fluid	Ovarian weight
	cc	mgm	mgm	mgm
100	1.0	225	110	80
50	0.5	213	84	57
50	0.5	260	101	35
25	0.5	208	105	30
25	0.5	190	84	36
12.5	0.5		22	11.2

* Volume of urine from which material injected was derived

has proved to be a serviceable estimate for making comparisons

The maximal daily variation in gonadotropic excretion was determined for several patients during long periods under the most varied conditions Figure 1 illustrates the results from a patient aged 71 years This case was chosen to illustrate daily variations because, first, it shows the most extreme variations ever seen in this laboratory and second, it was observed the longest, a little over 3 months The following variation in con-

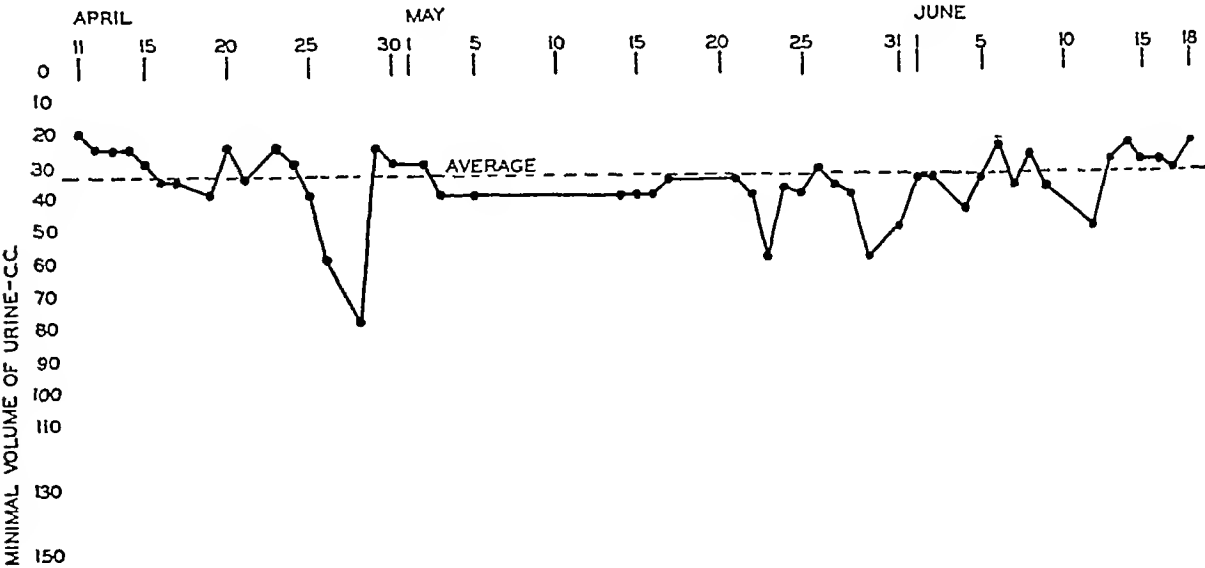


FIG. 1

DAILY VARIATION IN GONADOTROPIC HORMONE OUTPUT FOR A 71-YEAR-OLD POSTMENOPAUSAL WOMAN

The minimal volume of urine—cc. indicates the least amount of urine that had to be concentrated in order to stimulate uterine development in the rat.

ditions should be noted (1) A complete 24-hour urine specimen was not always secured (2) Refrigeration was omitted occasionally (3) Length of time between urine collection and precipitation varied from 1 day to 6 weeks—without any preservative, but with refrigeration (4) Length of time between precipitation and preparation of solution varied from 1 day to 2 months (5) Length of time between preparation of solutions and injection varied from 1 to 10 days (6) Responses of immature rats used are inherently variable to this natural variation can be added the factors of toxicity of some specimens and not of others, subcutaneous hematomas causing occasional leakage and finally the variation in laboratory skill attending the concentrating of the urine. From Figure 1 it can be seen that the average response was at 34.8 cc. equivalents, the minimal response at 20 cc. equivalents, and the maximal response at 80 cc. equivalents. In percentages the most extreme variation from the mean response over the 3-month period was 43 per cent in the case of the minimal and 130 per cent in the case of the maximal.

The low assays were more probably due to any or all of the above mentioned variations than the high assays. The only condition that might cause a false high assay is an incomplete specimen that contains the highly concentrated night urine. Therefore, in a practical interpretation of an individual result, more faith may be put in the higher assays.

In the above case no corrections were made for variations in volume (which were substantial) because of the known incompleteness of the collections. When and if completeness of collection is secured, a correlation can be found between volume and response. In general, it was observed that the response per unit volume varied inversely with the volume itself.

A study of the day-to-day variations, which in all other cases were smaller than those in the case discussed above, indicates that not more than 3 samples collected on consecutive days are needed to furnish a satisfactory estimate of gonadotropic hormone content of the urine from a given patient. To date, no indication of any cyclic variations in excretory activity during or after the menopause has been noted. Twelve-hour night specimens offer the advantage of originally supplying the gonadotropic hormone in a more concentrated form than the 24-hour specimens. They were therefore adopted for routine collections.

RESULTS AND DISCUSSION

Urines from 66 patients were concentrated and assayed as described. The ages of the patients ranged from 24 to 77 years.

From Figure 2 it can be seen that, when classed according to titer, the patients fall into three general classes. (1) Those whose urines showed very low gonadotropic potency were all from patients exhibiting *regular* normal cycles (2) Those whose urines ran the whole gamut of titers

from the lowest to the highest were from women having *irregular* cycles (3) Those whose urines were very high in potency were from women who have *ceased cycling* either spontaneously or artificially. The minimal volume of urine necessary to stimulate one rat was for each of the three groups 400 to 500 cc. 25 to 400 cc. and 10 to 30 cc., respectively.

It was observed that women having regular normal menstrual cycles excrete varying amounts of gonadotropic hormone during their cycle. However there is no day in the cycle when gonadotropic hormone is regularly absent from the urine, as is so frequently claimed. Usually there is a rise in titer between the 10th and 20th days. Therefore assaying urine during the mid interval was avoided when comparisons were being made between the potency of normal and menopausal women.

The assays answer the questions originally posed in the following manner:

(a) Concerning the relationship of age to gonadotropic potency a brief inspection of Table II shows that age is a factor only insofar as it influences the time of onset of the menopause. Women who have not yet reached the climacteric excrete little gonadotropic material, regardless of age. Women who have passed the climacteric have a high urinary gonadotropic output. This point can be illustrated by noticing that Patient Sf Class 6, a premature menopausal case, had a high concentration at age 25 years while an older non menopausal patient, Be Class 1, was still low in potency at age 48.

(b) Symptoms did not influence gonadotropic potency. Symptoms when used in this report will refer to both the autonomic and psychic symptoms of the menopause as described by Sevringhaus (7). Patients with symptoms who have ceased cycling, Class 6 have high titers. Patients without symptoms who have ceased cycling Class 5, have titers just as high. The series of 14 castrates (Classes 7 and 8) further substantiates the lack of correlation between symptoms and gonadotropic excretion. All 14 were almost exactly alike in potency although 10 had vasomotor symptoms and the other 4 had none. Those women who cycle regularly (Classes 1 and 2) and those cycling irregularly (3, 4)

TABLE II†
Gonadotropic hormone titer of women in the menopausal age period

Class	Cycling regularly									Cycling irregularly									
	1 No symptoms							2 Having symptoms			3 No symptoms	4 Having symptoms							
Patient	Mn	Wa	Ol	Ov	Be	Ca	Gn	Sw	Er	†By	Gl	†Tu	La	Po	Ne	Kr	He	Br	†Bn
Age years	43	44	33	47	48	45	43	52	45	44	45	42	42	36	46	38	45	46	49
Minimal volume of original urine cc	400	400	500	400	400	>300	400	>100	400	400	30	400	60	25	50	50	100	25	80
Class	Not cycling																		
	5 No symptoms										6 Having symptoms								
Patient	†St	†Th	Sm	Kn	Fr	Se	Sh	Me	Sk	Ma	Jo	Te	†Sa	Ol	Ra	Lt	*Gu	*Sd	Sf
Age years	58	59	77	48	51	57	67	73	66	71	52	27	47	58	49	42	57	56	25
Minimal volume of original urine cc	20	25	15	10	25	25	15	25	30	15	20	15	25	25	25	30	10	15	25
Class	Castrate													Hysterectomized					
	7 No symptoms				8 Having symptoms									9 Having symptoms					
Patient	Le	Ba	†Sl	†Ko	†Je	†Zu	†Vi	Gl	†Fh	Lg	Sr	Bl	†Da	Sc	Lh	Ve	Ho	Gu	Sd
Age years	52	47	47	24	47	43	33	40	29	39	36	46	29	43	46	36	43	57	56
Minimal volume of original urine cc	25	20	15	20	15	25	20	15	10	25	20	25	30	40	25	>200	40	10	15
Class	Involution																		
	10																		
Patient	Je	Lr	At	Fh	An	St	Th	Tu	Vi	Ve	Sl	Ko	Da	Zu	Bn	By			
Age years	47	48	39	29	50	58	59	42	33	36	47	24	29	43	49	44			
Minimal volume of original urine cc	15	200	275	10	10	20	25	400	25	>200	15	20	30	25	80	400			
Class	Estrin treated cases																		
	11 Showing improvement of symptoms																		
Patient	Ls	Kn	Sr	Lg	Sa	Vi	Bl	El	Sd	Ac	Fh	Pa	Fh	Ll	Bk				
Age years	31	48	36	39	47	33	56	42	56	49	29	52	29	49	51				
Minimal volume of original urine cc	40	10	25	25	25	50	40	30	20	40	20	10	20	10	50				

† Symptoms—Refers to the presence or absence of vasomotor phenomena at the time of titration

* Also classed as Hysterectomy

† Also classed as Involution

> indicates a negative reaction with the volume of urine used

tend to further the same argument, that there is no correlation between presence or absence of symptoms and gonadotropic titer. Similarly, there is no correlation between severity of symptoms and gonadotropic potency. For example, Patient Ba, Class 7, who had no symptoms, Patient Sr, Class 8, who had unusually severe symptoms, and Patient Bl, Class 8, who had mild symptoms all had the same gonadotropic concentration

(c) Many clinicians still retain the belief that at the menopause there is a greater gonadotropic

excretion than at any other time, although Österreicher (8), Saethre (9), and Jones and MacGregor (10) have already pointed out that senile women have a high gonadotropic excretion. Saethre (11) differs with Zondek (12), who reported negative findings in 85 per cent of postmenopausal women investigated. Classes 5 and 7 substantiate the observation that once the gonadotropic concentration rises at the time of the climacteric, it remains at the *same* high level throughout the duration of life. Neither the presence or

absence of symptoms at the time of the climacteric, nor the length of time elapsed since the climacteric seem to influence the gonadotropic excretion rate.

(d) There is no essential difference between the gonadotropic potency of urines from spontaneous and artificial menopause patients (see Classes 5, 6, 7, and 8 in Table II and Figure 2). The length of time elapsed since castration does not play an important role either, for example, Patient Le who had been a castrate for 20 years and Patient Ba who had been castrated only 6 months before, had the same potency (Class 7).

(e) There was no uniformity in gonadotropic assay in urines from patients diagnosed as having involuntional psychoses. But when these cases were reclassified according to the regularity, irregularity, or cessation of their menstrual cycles, their titers fitted in with those of the groups with which they were classed (see Class 10 and those marked † in the other classes).

(f) The hysterectomized patients (Class 9) had nothing in common with one another but seemed to titrate high or low according to whether or not they fell into the true menopause class. Since there could be no evidence of cyclic activity any reclassification is only a guess.

(g) The status of the menstrual cycle, *i.e.* regular, irregular, or absent, is the only factor which influences the urinary gonadotropic potency during the menopausal period. Classes 1 and 2, consisting of regularly cycling women with uneventful menstrual histories, contained uniformly low titers. From 400 to 500 cc. of urine was necessary to stimulate one rat. Classes 5, 6, 7, and 8, consisting of acyclic women of menopausal age or beyond, contain uniformly high titers 10 to 30 cc. being needed to stimulate one rat.

Between these two extremes lie Classes 3 and 4. These women all gave a history of having had regular cycles until recently, now they have become irregular in one way or another, *i.e.* in time, duration of flow, amount of flow, *etc.* The most common changes observed were lengthening of the cycle, skipping periods, and scantier flow. All but one were having mild or severe vasomotor symptoms. However, the majority had had symptoms and irregularities of the cycle for but a short time. Thus these women may be considered to be just entering the menopause, or in a transitional period between normal cycles and the

menopausal state. As is to be expected, the gonadotropic titers were in general neither as high as in the menopausal state or as low as in the normal state. However, titers equal to either of the other two classifications were encountered.

Thus, while there is a lack of correlation between gonadotropic titer and age, symptoms, hysterectomy and involution, there is a definite correlation between gonadotropic hormone titer and the presence or absence of the menstrual cycle in women of menopausal age.

(h) A striking observation about these assays is the tremendous difference between urines of normal and menopausal women. A 20-fold increase in gonadotropic potency occurs at the time of the climacteric. This increase develops in a relatively short time, probably 2 to 10 years. In order to definitely establish this individual cases should be followed through the menopausal period.

One of the practical applications of these findings is that premature menopause can be differentiated from the other amenorrheas. Two cases of amenorrhea in young women, Sf in Class 6 aged 25, and another patient aged 25 were recently studied. Patient Sf titrated high (25 cc.) and was classed as a premature menopause, while the other case proved to be completely negative in gonadotropic content at the 800 cc. level. This response is less than occurs in the normal since urine from normal women elicits positive responses at the 400 to 500 cc. level. These findings confirm the clinical diagnoses of premature menopause and hypopituitary amenorrhea, respectively.

Another practical clinical application, and perhaps the one of greatest importance, would be the use of the gonadotropic titer as a therapeutic index in treating menopausal symptoms with the estrogens. Albright (13), Frank and Salmon (14), Jones, MacGregor, and Tod (15), and others have reported decreases of gonadotropic excretion which occurred concurrently with the clinical improvement of the menopausal symptoms. Jones, MacGregor and Tod (15) found that certain refractory psychotic postmenopausal cases showed a reduction in gonadotropic potency, although they did not respond to estrogen therapy clinically.

We were therefore disappointed to find that

MINIMAL VOLUME OF URINE—CC.

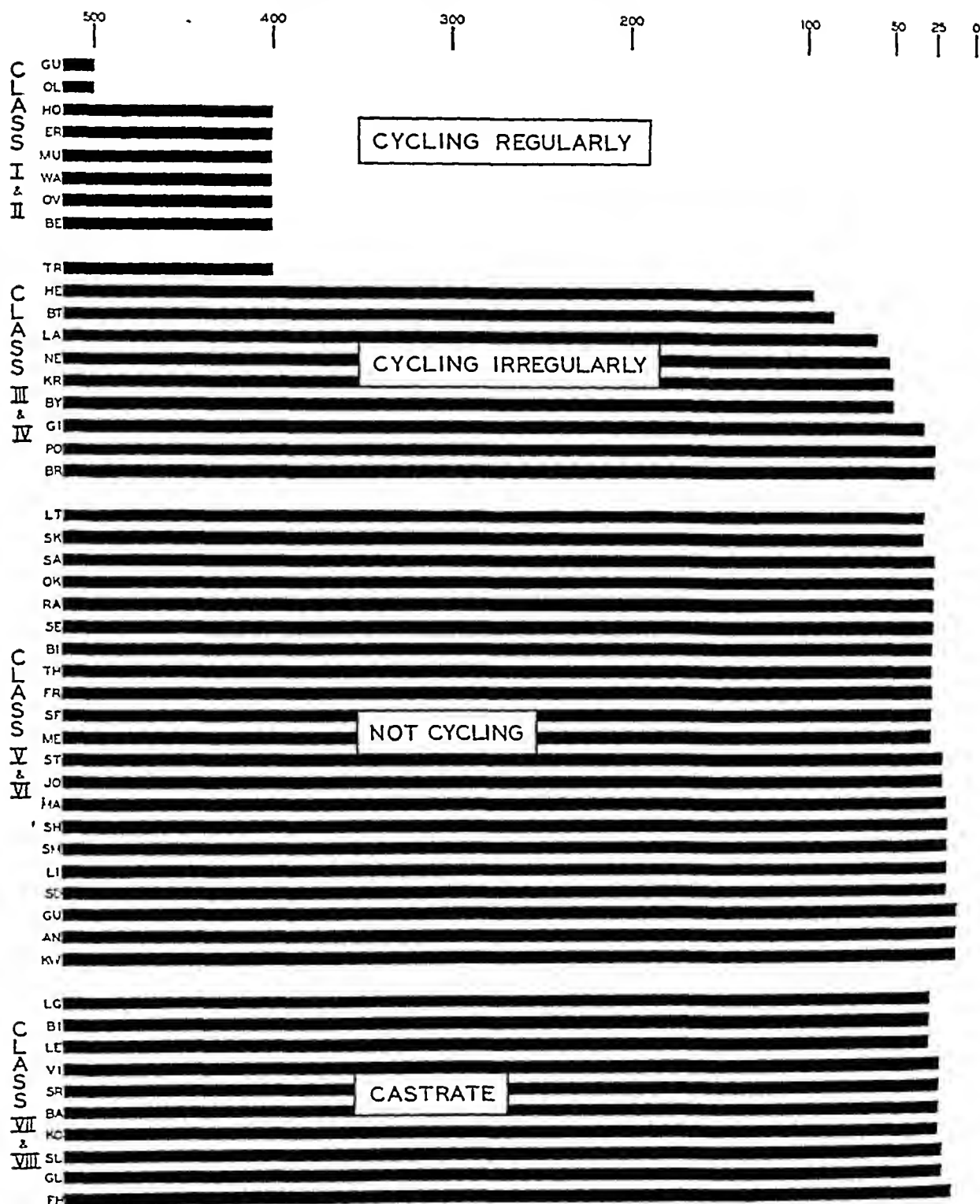


FIG. 2. THE GONADOTROPIC HORMONE EXCRETION OF MENOPAUSAL WOMEN

The minimal volume of urine—cc. indicates the least amount of urine that had to be concentrated in order to stimulate uterine development in the rat.

estrogen treatment² did not materially reduce gonadotropic potency (Class 11) in the 15 cases which we have discussed. In each instance estrogen therapy caused a definite clinical improvement. The amounts and methods of administration varied with the individual patient. Two preparations were used—Amniotin (Squibb) and Progynon DH (Schering). Amniotin, 2000 IU given orally four times a day was the usual maximum dose. In two refractory cases intramuscular injection of 10 000 IU daily was resorted to. Usually clinical relief was achieved in less than one month. Samples for assay were taken from one month to one year after therapy was instituted, and while it was continued.

The number of cases is small and the time of treatment is relatively short. When estrogenic therapy was continued for longer periods the gonadotropic potency was reduced to a 40 to 50 cc. minimal in a few cases. This is still far from the normal minimal of 400 to 500 cc.

The reports by earlier workers may be rationalized if one considers that only positive or negative stimulation of the assay animals was observed. An analysis of their data shows that their assays were probably made at the borderline of minimal stimulation. Therefore any slight change in potency might account for the negative findings reported in treated cases. Since these workers do not mention the gonadotropic titer of normally cycling asymptomatic women, or of acyclic asymptomatic menopausal women in the same age groups as patients with symptoms undue significance possibly may have been attached to a very slight decrease in gonadotropic potency.

From our results, it would seem that gonadotropic hormone is not the chief factor concerned with the symptoms of the menopause as Albright (13) and others suggested it might be. In proof of this we have offered the evidence, (1) that the titer of symptomless women can not be distinguished from those having symptoms (2) that estrin therapy at a point that adequately controlled the symptoms did not reduce the gonadotropic titer (although upon further treatment the

potency is sometimes slightly reduced), (3), the animal work recently reported by Lauson, Heller, and Sevringhaus (16). It was shown that estrogenic substitution, in doses which caused thymus atrophy, pituitary hypertrophy, and constant vaginal estrus did not materially reduce the pituitary potency of castrated female rats. Therefore, it is to be seriously questioned that estrogens can return the potency to normal without having the unfavorable or undesired side effects which occur in castrated rats.

Henderson and Rowlands (17) have recently assayed pituitaries obtained at autopsy from women of various ages. They found that the pituitaries from premenopausal individuals were low in gonadotropic potency, that an abrupt rise occurred at the time of the menopause, and that the high potency was maintained during senility. We have assayed the serum from 10 of the cases reported here. Ten cc. were injected in 1 cc. doses twice daily for 5 days. All serum from women who had low urinary gonadotropic potency was negative. Most of the serum from women who had a high urinary potency gave a positive gonadotropic reaction. So there appears to be a positive correlation between the gonadotropic titers from the pituitary gland, the blood serum, and the urine in the human female.

SUMMARY

Urnines from 66 menopausal patients were assayed for their gonadotropic potency. It was found that their potency was not related to the presence or absence of symptoms, age, hysterectomy, or the involution. No difference in gonadotropic potency was found between menopausal women with symptoms and senile women castrated women or menopausal women without symptoms.

Urinary gonadotropic concentration was low in women with *regular* normal menstrual cycles, high in menopausal women in whom cycles had *ceased*, and intermediate in menopausal women with *irregular* cycles.

Estrogen treatment alleviated the vasomotor symptoms of 15 menopausal women but failed to concurrently reduce their gonadotropic potency. Continued estrogen therapy caused a slight reduction in potency but failed to suppress it by significant amounts.

²The estrogen treated patients were made available for this study through the kindly cooperation of Dr. E. L. Sevringhaus and Dr. E. S. Gordon of the Department of Medicine and of Dr. M. J. Musser of the Department of Neuropsychiatry.

We wish to express our appreciation for the diligent help given us by Arthur Bleecker and Lewis Aasen in making the assays

BIBLIOGRAPHY

- 1 Novak, E., Discussion following papers read at the Section on Obstetrics, Gynecology, and Abdominal Surgery, at the Eighty-Eighth Annual Session of the American Medical Association. *J A M A*, 1937, 109, 1877
- 2 Sevringhaus, E. L., *Ibid J A. M A*, 1937, 109, 1878
- 3 Levin, L., and Tyndale, H. H., The quantitative assay of "follicle-stimulating" substances *Endocrinology*, 1937, 21, 619
- 4 Heller, C. G., Lauson, H., and Sevringhaus, E. L., The immature rat uterus as an assay end-point for gonadotropic substances *Am. J Physiol*, 1938, 121, 364
- 5 Levin, L., and Tyndale, H. H., Concentration and purification of the gonadotropic substance in urine of ovariectomized and post-menopausal women. *Proc. Soc. Exper Biol. and Med*, 1936, 34, 516
- 6 Heller, C. G., and Heller, E. J., Gonadotropic hormone—clinical application of extraction methods for assay purposes *Endocrinology*, 1939 (In press)
- 7 Sevringhaus, E. L., The menopause Diagnostic and therapeutic problems *Southwestern Med.*, 1938, 22, 128
- 8 Österreicher, W., Vermehrte Ausscheidung von Hypophysenvorderlappenhormon (Prolan) im Harn in Der Involutionsperiode BZW im Senium. *Klin Wchnschr*, 1932, 11, 813
- 9 Saethre, H., Titrierung von Sexualhormonen Bei Geisteskranken. *Klin. Wchnschr*, 1933, 12, 1409
- 10 Jones, M. S., and MacGregor, T. N., Inhibitory effect of follicular hormone on the anterior pituitary in humans *Lancet*, 1936, 2, 974
- 11 Saethre, H., Über die Ausscheidung von Prolan Im Harn In Der Involutionsperiode BZW Im Senium. *Klin. Wchnschr*, 1933, 12, 1727
- 12 Zondek, B., Die Hormone des Ovarium und des Hypophysenvorderlappens Julius Springer, Berlin, 1931, 1st ed.
- 13 Albright, F., Studies on ovarian dysfunction III The menopause. *Endocrinology*, 1936, 20, 24
- 14 Frank, R. T., and Salmon, U. J., Effect of administration of estrogenic factor upon hypophyseal hyperactivity in the menopause. *Proc. Soc. Exper Biol and Med.*, 1935, 33, 311
- 15 Jones, M. S., MacGregor, T. N., and Tod, H., Oestradiol benzoate therapy in depressions at the menopause. *Lancet*, 1937, 1, 320
- 16 Lauson, H., Heller, C. G., and Sevringhaus, E. L., Inadequacies of estradiol substitution in ovariectomized albino rats *Endocrinology*, 1938, 23, 479
- 17 Henderson, W. R., and Rowlands, I. W., The gonadotropic activity of the anterior pituitary gland in relation to increased intracranial pressure *Brit. M J*, 1938, 1, 1094

THE CONTROL OF METHEMOGLOBINEMIA WITH METHYLENE BLUE^{1,2}

By WILLIAM B. WENDEL

(From the Department of Internal Medicine Washington University School of Medicine St. Louis and the Department of Chemistry University of Tennessee College of Medicine Memphis)

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The cyanosis seen in a high percentage of patients receiving sulfanilamide and related drugs has been attributed variously to methemoglobinemia (1, 2), sulfhemoglobinemia (1), an unusual degree of unsaturation of the venous blood (3), and to the presence of aniline black (4) and other colored derivatives of sulfanilamide (5) in the red blood corpuscles. Marshall and Walz (4) and Chesley (6) consider methemoglobin to be of no importance except perhaps in an occasional patient.

The present paper summarizes the results of determinations of methemoglobin in the blood of more than one hundred patients who were receiving sulfanilamide and showing cyanosis. Methemoglobin was found at least in traces in over 90 per cent of these. In thirty five cases the blood contained methemoglobin to the extent of 15 per cent or more of the total pigment. In one instance 40 per cent of the total blood pigment was in the form of methemoglobin.

Animal experiments are described which confirm and extend the observations of Williams and Challis (7), Steele and Spink (8), and Hauschild (9) that methylene blue hastens disappearance of methemoglobin from the blood. Clinical application of these experiments to patients showing cyanosis from sulfanilamide has shown that intravenous injection of 0.1 to 0.2 cc. of one per cent methylene blue per kilogram body weight reduces

the concentration of methemoglobin from levels as high as 25 to 40 per cent of the total pigment to less than 4 per cent in 30 to 40 minutes.

In one case of sulfhemoglobinemia methylene blue was without effect upon the abnormal pigment.

ANIMAL OBSERVATIONS

Since, in our experience, sulfanilamide does not produce methemoglobin in dogs, rabbits, rats, and mice, nitrite was used to produce experimental methemoglobinemia. Figure 1 illustrates the course of blood pigment changes in seven control dogs which received intravenous injections of 30 mgm. of sodium nitrite per kilogram of body weight. Following injection of nitrite, methemoglobin³ rapidly accumulates and after 60 to 100 minutes reaches a maximum concentration corresponding to a loss of 60 to 70 per cent of the normal oxygen capacity. It then progressively decreases due to reconversion to hemoglobin, and after 8 to 9 hours has entirely disappeared. The average maximum rate of regeneration of hemoglobin from methemoglobin (indicated in Figure 1 by a broken line) in these seven animals was 0.03 volumes per cent per minute. This is essentially the same rate as observed when blood containing methemoglobin from these animals is incubated at body temperature *in vitro* and may be looked upon as the rate at which the enzyme systems in the erythrocytes are able to reduce methemoglobin (10).

¹ A preliminary report of this work was given before the Middle Section of the American Laryngological Rhinological, and Otolological Society St. Louis, January 26 1938, and the American Society of Biological Chemists Baltimore, March 30 1938 (J. Biol. Chem., 1938, 123 cxxiv) and was contained in a letter to the Editor of the J. A. M. A. 1937 109 1216.

² This work was supported at both schools by grants from the Upjohn Company Kalamazoo Michigan. The Upjohn Company has supplied the writer with 10 cc. ampoules of methylene blue which were used in the clinical studies.

³ Methemoglobin concentration is expressed in this paper in two ways, as per cent of the total pigment and as volumes per cent, one volume per cent of methemoglobin being that concentration which results from loss of one volume per cent oxygen capacity.

In most experiments methemoglobin was determined by a spectroscopic method which will appear in a forthcoming number of the J. Lab. and Clin. Med. Occasionally methemoglobin was determined also by difference between total pigment and oxygen capacity. The two methods usually gave identical results.

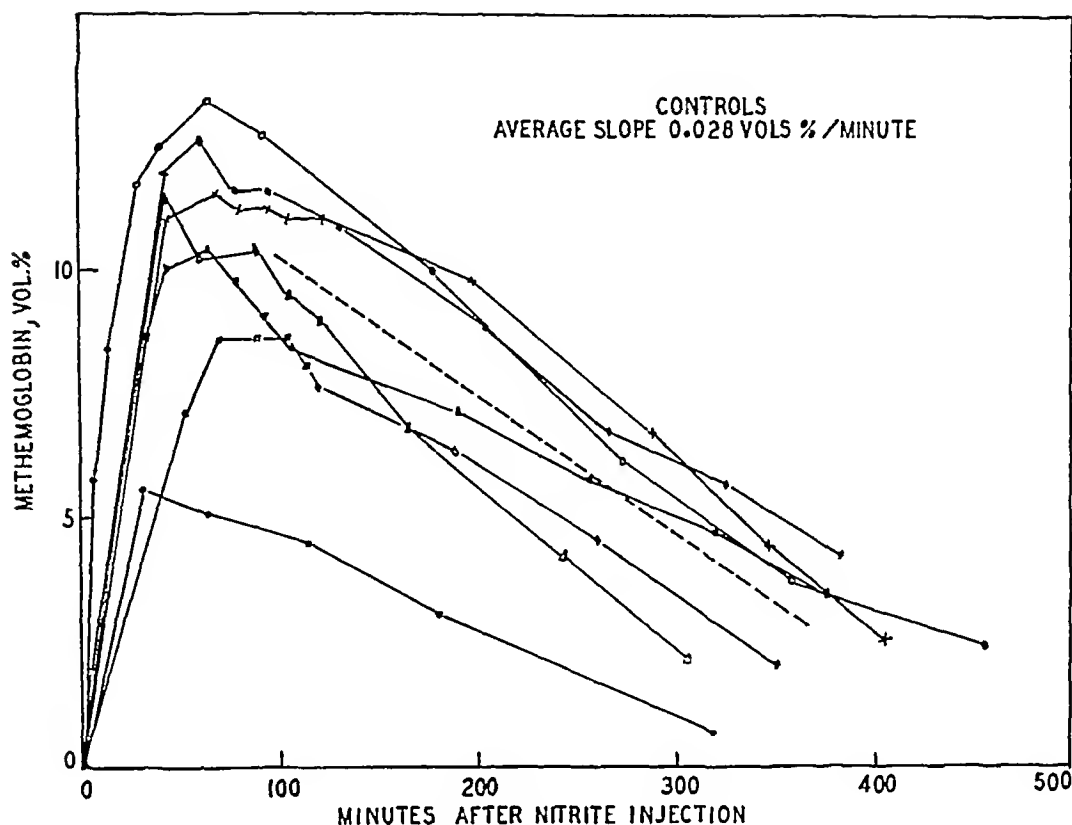


FIG 1 ACCUMULATION AND DISAPPEARANCE OF METHEMOGLOBIN IN THE BLOOD OF DOGS FOLLOWING THE INTRAVENOUS INJECTION OF SODIUM NITRITE (30 MG/M PER KILOGRAM OF BODY WEIGHT)

Having determined the physiological rate of hemoglobin regeneration from methemoglobin various substances were tested for possible accelerating action upon this process. Sodium formaldehyde sulfoxylate, which reduces methemoglobin in aqueous solution, was found to be without effect *in vivo*. Sodium formaldehyde sulfoxylate, however, reduces methylene blue and since reduced (leuco) methylene blue converts methemoglobin to hemoglobin very rapidly, it seemed possible that simultaneous injection of these two substances might accelerate conversion of methemoglobin to hemoglobin. A cycle between sulfoxylate in the plasma and methemoglobin in the red cells mediated by methylene blue was pictured. When tested it was found that simultaneous intravenous injection of methylene blue and sulfoxylate caused methemoglobin to disappear from the blood very rapidly. Control experiments, however, showed that methylene blue alone was equally effective. Figure 2, which illustrates the results

obtained with a number of dogs, shows that injection of 2 mgm of methylene blue (in the form of a one per cent aqueous solution) per kilogram of body weight increases the rate of disappearance of methemoglobin from the blood four or five-fold. Table I summarizes the results of a series of similar experiments in which the effect of various quantities of methylene blue upon the rate of disappearance of methemoglobin from the blood was determined. A measurable effect is evident even with the smallest amount of dye injected, namely, 0.1 mgm per kilogram of body weight. Oxygen capacity figures in Table I indicate that the methemoglobin which disappears rapidly following injection of methylene blue is converted to functionally active hemoglobin. The duration of the anti-methemoglobin action of 5 mgm of methylene blue per kilogram in dogs is at most about 2 hours.

Methylene blue accelerates the disappearance of methemoglobin also from the blood of rabbits

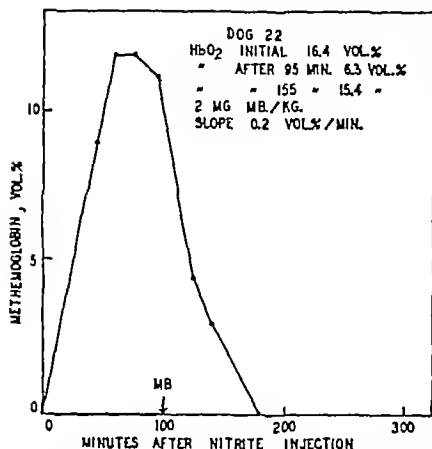


FIG. 2. EFFECT OF INTRAVENOUS INJECTION OF METHYLENE BLUE, 2 MG. PER KILOGRAM OF BODY WEIGHT ON RATE OF CONVERSION OF METHEMOGLOBIN TO HEMOGLOBIN IN THE DOG

HbO₂ means oxygen capacity MB over arrow indicates injection of methylene blue.

CLINICAL APPLICATION

In collaboration with Dr. Alexis F. Hartmann of Washington University Medical School, where the writer began and completed an important part of this work, these observations received first clinical application in the summer of 1937. Since these early observations many others have been made with the cooperation of physicians on the staff of the University of Tennessee Medical School. In summary intravenous injection of 0.1 to 0.2 cc. per kilogram of body weight of a one per cent aqueous solution of methylene blue converts in the course of about 45 minutes all of the methemoglobin in the circulating erythrocytes into functionally active hemoglobin, even when the methemoglobin concentration represents initially as much as 25 to 40 per cent of the total pigment. The rate at which methemoglobin reappears following injection of the dye varies with different individuals, but as a general rule the concentration of methemoglobin will have returned to its pre-methylene blue level not sooner than 12 hours and not later than 24 hours after injection of 2 mgm. of dye per kilogram of body weight.

Methylene blue is effective as an anti-methemoglobin agent also when given by mouth. As compared with intravenous injection the response to administration by mouth is slow, and more of the dye is required. Observations to date indicate that accumulation of methemoglobin can be prevented in adults by oral administration of 0.5 to 1.0 gram of methylene blue per day even when the patient is receiving large doses of sulfanilamide.

Before methylene blue was injected into patients receiving sulfanilamide, compatibility of these two substances was tested in dogs and rabbits. Several times as much methylene blue was injected into experimental animals as is required in humans. Also the dye was injected at a time when the animals were severely toxic from large doses of sulfanilamide (1 gram per kilogram per day). No increase in toxicity was evident following intravenous injection of the dye. In order to elicit possible cumulative effects of administration of the combination of drugs one dog was given 0.6 gram sulfanilamide per kilogram per day and 0.27 gram of methylene blue per day by mouth for 4 weeks. No outward evidence of toxicity or abnormal blood changes was observed.

Preliminary experiments with Dr. Anna Dulaney of the University of Tennessee Medical School on mice injected with highly virulent strains of beta hemolytic streptococci indicate that

TABLE I

Effect of various amounts of methylene blue on rate of conversion of methemoglobin to hemoglobin in dogs

Dog number	Amount of methylene blue injected	Rate of disappearance of methemoglobin	Oxygen capacity		
			Before nitrite	After nitrite	After methylene blue
	mgm. per kilogram	ml. per cent per minute	ml. per cent	ml. per cent	ml. per cent
14	10	0.77			17.2 (50 min)
14	10	0.831			18.5 (30 min)
21	10	0.811	12.8	4.4	
18	5	0.19			
18	9	0.25			
17	2	0.25	17.7	7.2	17.0 (45 min)
22	2	0.20			
22	2	0.20	16.4	8.3	15.4 (50 min)
19	1	0.076	12.7	5.5	10.6 (45 min)
20	1	0.14			
6	0.5	0.12			
17	0.5	0.083			
22	0.1	0.048			
20	0.1	0.021			
Five control animals	0	Average=0.025			

* Determined at peak of methemoglobin formation.

† Figures in parentheses denote interval between injecting methylene blue and determining oxygen capacity.

‡ Rate was actually faster than this. Method did not permit accurate determination.

methylene blue does not interfere with the therapeutic action of sulfanilamide in these animals

Sulfanilamide methemoglobinemia

The frequency of methemoglobinemia in patients receiving sulfanilamide has not been determined. We have, however, examined for the presence of methemoglobin the blood of more than one hundred patients who were showing cyanosis from this drug. In this group of patients where the blood was examined within 30 minutes after being drawn and the sulfanilamide concentration was greater than 4 mgm. per cent, methemoglobin was found at least in traces (more than 3 per cent of the total pigment) in every blood except two. Table II summarizes data obtained on samples of blood containing more than 15 per cent of methemoglobin and whose sulfanilamide concentration also was determined (11). Most of these samples of blood were from patients who were receiving 0.1 to 0.2 gram of sulfanilamide per kilogram of body weight per day in divided doses at 4-hour intervals. In each case the dose of sulfanilamide had been constant 24 hours prior to collection, in most, it had been constant 48 hours. Where several sets of data are given on one patient, they represent analyses of samples of blood taken on different days, sometimes at widely separated intervals and following change in dosage. No correlation between concentration of sulfanilamide in the blood and intensity of methemoglobinemia is evident in these data.

Evidence that methemoglobin is the principal abnormal pigment in the blood of patients showing cyanosis from sulfanilamide will be published in detail elsewhere but may be summarized as follows: (a) When a freshly drawn sample of blood from such a patient is laked with 4 or 5 volumes of water in a tube of about one inch in diameter and is examined with a hand spectroscope before a 60 to 100 watt frosted light bulb, it usually shows an absorption band in the red region of the spectrum (at $\lambda = 630 \text{ m}\mu$). In a given sample of blood the intensity of this band is approximately the same as that calculated for methemoglobin from the difference between total pigment and oxygen capacity, this difference being assumed to be owing to methemoglobin alone. Following addition of a buffer solution of pH 6.5 this band is intensified. Addition of sodium

TABLE 11

Lack of relationship between concentration of sulfanilamide in the blood and the degree of methemoglobinemia

Patient	Blood sulfanilamide	Methemoglobin	Total pigment
	mgm per cent	per cent of total pigment	volumes per cent
L F	4 4	22 16	7.8 7.8
H L	34 50 27 20 17	33 25 27 22 20	14.6
E B	8	29	13.4
L M	14	17	18.1
W R. S	14	29	15.1
M A.	5 6	21 29	16.5
B D	20	25	6.4
L McM	7 5	22 17	11.9 12.4
E W	26 10 12	30 30 23	14.4
E J	6 5	22 25	22.0
T	5	23	13.9
Y	20 9	25 25	
H I	5	25	11.0
J T	9 8	33 33	
C B	6	20	
B T	3 10 8 6	13 30 33 25	
M L M	5	28	
G O	8	27	18.1
R. L. L	14	29	14.0
B S M	3	26	
O B S	7	22	
B	12 7	27 30	14.2

cyanide or carbonate dissipates it entirely. In an occasional sample of blood there is an absorption band in the red portion of the spectrum (some-

what nearer the yellow) which is unaffected by alkali or cyanide and which is caused by the presence of sulfhemoglobin. The following statements therefore, are intended to apply only to those cases in which sulfhemoglobin is absent. (b) The absorption band in the red region of the spectrum is greatly weakened or is entirely dissipated by injection of methylene blue. At the same time the color of the blood changes from reddish brown to a more normal red. Also following injection of methylene blue the oxygen capacity of the blood increases and usually becomes equal to the total pigment concentration (determined spectrophotometrically as cyanmethemoglobin). This important point is illustrated by the data in Table III. (c) Spectrophotometric

TABLE III
Increase in oxygen capacity of blood of patients following intravenous injection of methylene blue

Patient	Blood sulfanilamide	Total pigment	Oxygen capacity	Difference between total pigment and oxygen capacity	Methemoglobin (By spectroscopic method)	Remarks
H. L.	23	18.4 12.5	10.8 12.4	8.1 0.1	2.3 0.3	Before MB 1 hour after 18 cc. 1 per cent MB
	23	12.5 12.6			2.7 0	Before MB 80 minutes after 10 cc. 1 per cent MB
L. M.	14	20.5	18.9 18.6	5.4	2.6	Before MB 24 minutes after 15 cc. 1 per cent MB
W. R. B.	14	15.1	10.4 12.8	4.7	4.4 0.7	Before MB 48 minutes after 2 cc. 1 per cent MB
M. A.	6	16.5 16.5	13.8 16.0	2.7 0.6	4.8 0.6	Before MB 40 minutes after 10 cc. 1 per cent MB
H. L.	5	11.0 11.0	9.0 10.6	2.0 0.4	2.7 0.3	Before MB 70 minutes after 10 cc. 1 per cent MB
R. L. L.	14	12.0 11.7	9.4 11.1	2.6 0.6	2.8 0.4	Before MB 60 minutes after 5 cc. 1 per cent MB

* Concentration previous day
† MB = methylene blue.

measurement of the visible absorption spectrum indicates that oxyhemoglobin and methemoglobin are the only colored substances present in significant amounts in the blood of these patients. Such observations lend no support to the suggestion of Ottenberg and Fox (5) that the dark color of the blood from patients treated with sulfanilamide is owing *per se* to adsorption by the

erythrocytes of a colored derivative of sulfanilamide, formed by irradiation of dilute solutions of the latter. It is interesting to note, however, that the colored derivatives of sulfanilamide described by these workers convert hemoglobin to methemoglobin *in vitro*.

DISCUSSION

In 1933, Williams and Challis (7) and Steele and Spink (8) reported that intravenous injection of methylene blue into three patients showing severe poisoning and methemoglobinemia resulting from aniline and aniline derivatives brought about rapid recovery of the patients. Both groups of workers stated that the methemoglobinemia shown by their patients rapidly subsided following injection of the dye. These observations did not receive the attention they deserved probably because of the emphasis which was placed at that time upon the reverse transformation which accounts for the antidotal action of methylene blue in cyanide poisoning (12). Also the spectroscopic data of Williams and Challis (7) were not altogether convincing. Nevertheless, their conclusions were correct, and it is necessary to explain how methylene blue may either convert hemoglobin to methemoglobin and thus act as an antidote for cyanide or hasten the reverse transformation and act as an antidote for methemoglobin forming substances.

Methylene blue forms methemoglobin by catalytic oxidation (10). The dye reacts directly with hemoglobin to form methemoglobin, and the reduced (leuco) form of methylene blue formed by this reaction reacts with oxygen to regenerate methylene blue. It is not equally clear how methylene blue accomplishes reduction of methemoglobin to hemoglobin. Since each molecule of dye injected effects conversion of many molecules of methemoglobin to hemoglobin this reaction too, is catalytic. Here however, the catalysis is one of reduction, and leuco methylene blue would appear to be the effective reductant. Two possible sources of the leuco methylene blue in the body are reduction of methylene blue in the erythrocytes by enzyme systems present there and reduction of methylene blue in other tissues. Preliminary experiments suggest that the rate of formation of leuco methylene blue in the erythrocytes may not be sufficiently rapid to account for

all the methemoglobin reduced. Thus it would appear that leuco methylene blue formed in the more actively metabolizing tissues and returned as such to the erythrocytes may play a rôle in reducing methemoglobin to hemoglobin. Experiments designed to test this possibility are in progress.

Owing to the limited time during which methylene blue has been used with sulfanilamide, its clinical value as an adjunct to sulfanilamide therapy can not as yet be assessed. It seems possible that the use of sulfanilamide might be less hazardous in many patients, especially those having respiratory and cardiac involvements, if the accumulation of methemoglobin were prevented. Also, the usefulness of sulfanilamide might be extended by giving larger doses, the methemoglobinemia being controlled by methylene blue.

In conclusion it should be borne in mind that intravenous injection of methylene blue may not be entirely without ill effects. Gregoire (13) has reported experiments on rats which suggest that patients kept in oxygen tents should be given methylene blue intravenously only with caution. Nadler, Green, and Rosenbaum (14), on the basis of electrocardiographic studies, state that methylene blue depresses the ventricular musculature and warn against its indiscriminate use. Also, hemolytic anemia has been observed in dogs following intravenous injection of this dye (15). These reports of toxic effects, however, were associated with much greater amounts of methylene blue (20 to 50 mgm per kilogram of body weight) than is required to control sulfanilamide methemoglobinemia. Nevertheless, even small amounts of the dye should be injected carefully and slowly. Leakage of the dye around the vein may produce a painful infiltration, and too rapid injection may produce hives and a severe burning sensation of the lips.

SUMMARY

The principal abnormal pigment in the blood of patients showing cyanosis from sulfanilamide appears to be methemoglobin.

Patients whose functionally active blood pigment is decreased 15 to 30 per cent by formation and accumulation of methemoglobin are not uncommon. Higher concentrations of methemoglobin are occasionally encountered.

The extent to which methemoglobin accumulates is not proportional to the concentration of sulfanilamide in the blood.

Following intravenous injection of small amounts of methylene blue, methemoglobin rapidly disappears from the blood and is replaced by an equivalent amount of hemoglobin.

Observations on animals which preceded the clinical ones are described. A possible mechanism by which methylene blue accomplishes conversion of methemoglobin to hemoglobin is outlined.

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BIBLIOGRAPHY

- 1 Paton, J P J, and Eaton, J C, Sulfhemoglobinemia and methemoglobinemia following administration of *p*-aminobenzenesulfonamide. *Lancet*, 1937, 1, 1159.
- 2 Bensley, E H, and Ross, J B, Methemoglobinemia due to sulfanilamide therapy. *Canad. M A J*, 1937, 37, 62.
- 3 Mull, J W, and Smith, J T, Effect of sulfanilamide on the oxygen capacity of the blood. *J A M A*, 1938, 110, 439.
- 4 Marshall, E. K., Jr, and Walzl, E. M, On the cyanosis from sulfanilamide. *Bull Johns Hopkins Hosp*, 1937, 61, 140.
- 5 Ottenberg, R., and Fox, C L., Jr, Explanation for the cyanosis of sulfanilamide therapy. *Proc. Soc. Exper Biol. and Med*, 1938, 38, 479.
- 6 Chesley, L C, Cyanosis without sulf- or methemoglobinemia in patients receiving sulfanilamide treatment. *J Clin. Invest.*, 1938, 17, 445.
- 7 Williams, J R., and Challis, F E., Methylene blue as an antidote for aniline dye poisoning. *J Lab and Clin Med.*, 1933, 19, 166.
- 8 Steele, C. W., and Spink, W W, Methylene blue in the treatment of poisonings associated with methemoglobinemia. *New England J Med*, 1933, 208, 1152.
- 9 Hauschild, F, Die Wirkung des Katalysins (Thionin) bei der Methämoglobinvergiftung. *Arch. f exper Path. u. Pharmacol.*, 1937, 184, 458.
- 10 Warburg, O, Kubowitz, F, and Christian, W., Über die katalytische Wirkung von Methylenblau in lebenden Zellen. *Biochem. Ztschr*, 1930, 227, 245.
- 11 Marshall, E. K., Jr, Determination of sulfanilamide in blood and urine. *Proc. Soc. Exper Biol and Med*, 1937, 36, 422.
- 12 Wendel, W B., Methylene blue, methemoglobin, and cyanide poisoning. *J Pharmacol. and Exper Therap.*, 1935, 54, 283.

- Chen K. K., Rose, C. L., and Clowes G. H. A., Comparative values of several antidotes in cyanide poisoning. *Am. J. M. Sc.* 1934 188 767
13. Gregoire, P. E., Action of methylene blue on body temperature and metabolism. *J. Exper. Med.*, 1931 54, 827
14. Nadler J. E., Green, H., and Rosenbaum, A., In travenous injection of methylene blue in man, with reference to its toxic symptoms and effect on the electrocardiogram. *Am. J. M. Sc.*, 1934 188 15
15. Huyghebaert, E., Action hémolytique du bleu de méthylène. *Arch. Internat. de pharmacodyn. et de therap.*, 1924 29 405
- Wendel W. B. and Hefley M. L., Methylene blue as an agent for reducing red blood cell count. *Proc. Soc. Exper. Biol. and Med.*, 1934 31, 973

ADDENDUM

Since this paper was accepted for publication the method used for methemoglobin determination has been described (*J. Lab. and Clin. Med.*, 1938, 24 96)

Also Hartmann Perley and Barnett (*J. Clin. Invest.*, 1938, 17 699) have published results of their observations on methemoglobinemia resulting from sulfanilamide, and the effect of methylene blue upon this

WATER EXCHANGE OF PREMATURE INFANTS—COMPARISON OF METABOLIC (ORGANIC) AND ELECTROLYTE (IN-ORGANIC) METHODS OF MEASUREMENT^{1, 2}

By HARRY H. GORDON, SAMUEL Z. LEVINE, ELEANOR MARPLES
HELEN McNAMARA, AND HELEN R. BENJAMIN

(From the New York Hospital and the Department of Pediatrics, Cornell University Medical College, New York City)

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In 1923 Gamble, Ross, and Tisdall (1) presented data correlating the fixed base and water balance of two fasting epileptic children. In these observations, the water balance was represented as the difference between total body weight loss and the sum of the protein and fat loss; the latter calculated from the urinary nitrogen and ketones. In 1929 Newburgh (2) and his coworkers described a method for the calculation of the water exchange of normal adults which depended on a knowledge of the total caloric expenditure and the composition of the metabolic mixture. The limitations of this so-called metabolic method have been discussed in detail for adults by Lavietes (3), Peters (4), and Newburgh *et al.* (5) and for infants by Levine *et al.* (6). In 1935 Lavietes, D'Esopo, and Harrison (7) proposed a method for estimating water balance from base balance and changes in concentration of base in serum. This method was suggested by the earlier observations of Gamble and his coworkers (1) that water and base are lost from the body in approximately the proportions in which they appear in body fluids. In recent years the "electrolyte" method for measuring water exchange has been applied, particularly by Darrow, Yannet, and Harrison (8) and Hastings and his coworkers (9), to quantitative studies of the relative distribution of intracellular and extracellular water.

Both the "metabolic" and the "electrolyte" methods of measuring total water exchange are open to criticism on theoretical grounds since the former assumes that the respiratory exchange is a reliable index of the composition of the metabolic mixture and the latter that certain electrolytes are retained solely as extracellular or intra-

cellular components in uniform concentrations throughout the body. Moreover, both methods are subject to errors in application in the presence of perceptible perspiration. In the organic method perspiration may interfere with prediction of the total calories from the insensible water loss, in the electrolyte method unmeasured skin excretion will give falsely high values for retention of minerals.

This report presents concurrent measurements of the water, organic, and electrolyte balances of four premature infants which permit a critical comparison of the above methods for determining total water exchange. Premature infants are particularly suited for such studies since interference by visible perspiration is reduced to a minimum. This tendency to minimal perspiration is dependent on poorly developed sweat glands, relatively large surface area for loss of heat by radiation, scanty adipose tissue and feeble muscular movements.

METHODS

Four healthy premature male infants were studied in eight observations of from 3 to 11 days for a total of 48 days. The infants ranged from 17 to 31 days and from 1900 to 2600 grams at the onset of observations. They resided in a constant temperature and humidity room and were exposed in customary clothing to environmental temperatures of from 72 to 87° F. and to relative humidities of from 40 to 80 per cent in the different observations. No difficulty was encountered in maintaining body temperature.

Diet

All food mixtures were prepared by the nurse in charge and were fed to the infants by trained assistants. The infants took their feedings well; their lips and the inside of nipples were wiped with sterile gauze which was immediately placed in weighed sealed jars to provide a quantitative estimate of the portion of the formula not swallowed. In these observations an average of 5 to 10 per cent of the initial formula adhered to the sides

¹Respiratory Metabolism in Infancy and Childhood XXII

²Assistance in this work was given by the Children's Bureau, U. S. Department of Labor

of utensils, bottles, bottle caps, and nipples, or was regurgitated. The amount actually ingested and retained was determined in each observation.

The diets consisted of boiled human milk, evaporated or powdered cow's milk to which water, dextrimaltose, olive oil, casein or calcium paracaseinate were added in varying amounts in the different observations. The caloric fluid and protein intake were adequate to permit satisfactory weight gains of 25 to 49 grams daily. Adequate concentrates of vitamins C and D were added to each infant's diet.

Thoroughly mixed samples of the powdered preparations and pooled daily aliquot samples of the liquid cow's and human milk were analysed for water, nitrogen, chloride, potassium, sodium, calcium, and phosphorus, and where indicated for fat and carbohydrate. All analyses were made in triplicate, the methods used are given at the end of the report.

Urine and feces

Urine and feces were collected separately and analysed for water, nitrogen, chloride, potassium, sodium, and phosphorus. Fecal calcium and fat were determined, and for Infants L. O. and E. M., urinary calcium as well.

Changes in body weight and total insensible perspiration

The infants were weighed daily with a balance having a capacity of 10 kgm. and an accuracy of 0.05 gram (10). The weight of the total insensible perspiration was determined for each 24 hours by subtracting from the total weight of the intake the sum of the weights of urine and feces and the change in body weight. The water lost through the skin and lungs was obtained by subtracting from the total insensible loss the portion due to $\text{CO}_2 - \text{O}_2$, calculated from the metabolic mixture (2). In two observations (7 and 8 on Infants L. O. and H. L., Table II) a filter paper (11) was applied for 60 seconds to the forehead and abdomen one to three times daily,

and then exposed to silver nitrate and sunlight as an index of chloride excretion from the skin.³

Total caloric expenditure and composition of metabolic mixture

The total caloric expenditure was determined for Infants L. O. and H. L. by combining 24-hour minute to minute records of activity with calorimeter observations of 2 to 5 hours made in a small respiratory chamber attached to a Benedict universal respiration table (12). The total caloric expenditure for Infants N. O. and E. M. was predicted from the insensible perspiration on the assumption that approximately 25 per cent of the total heat production of premature infants (13) is lost by vaporization of water. While the latter is admittedly not an ideal method of estimating total calories, the diminished tendency of premature infants to perspire and their low activity permits a satisfactory approximation, especially under constant environmental conditions. Furthermore, because of their low total caloric expenditure (150 to 200 calories per 24 hours) an error of as much as 20 per cent in prediction would introduce a maximum deviation of only 4 to 5 grams in the daily water balance.

The urinary nitrogen was used as a measure of protein catabolism and the dietary carbohydrate as a measure of carbohydrate combustion (2, 14). The calories derived from fat were obtained by subtracting the protein and carbohydrate calories from the total.

Methods of calculating water balance

The methods used for calculating the water balance are presented in Table I. According to the direct or—

³ This procedure has now been made routine in all mineral balance observations, and whenever the test proves positive the room temperature is lowered or clothing is removed to prevent appreciable excretion of chloride through the skin.

TABLE I
Methods of estimating water balance

"ORGANIC" METHODS

Direct (2)		Indirect	
Water intake	minus	Water output	= Water balance
1 Ingested as such		1 Urine	= Total body weight change minus change in weight due to solids Protein = Intake minus urine, feces Fat = Intake minus feces, metabolized (CHO) = Intake minus metabolized (Minerals) = Intake minus urine, feces (skin)
2 In solid foods		2 Feces	
3 Oxidation		3 Skin and lungs	
Protein $\times 0.41$		$\text{H}_2\text{O} = \text{I.L.} - (\text{CO}_2 - \text{O}_2)$	
Fat $\times 1.07$		$\text{CO}_2 - \text{O}_2 = (\text{P} \times 0.08)$	
CHO $\times 0.60$		$-(\text{F} \times 0.08) + (\text{C} \times 0.41)$	

"ELECTROLYTE" METHODS

Anion		Cation (7)	
$\frac{\text{Chloride retained, mM}}{120} \times 1000$	= Extracellular H ₂ O grams (8d)		
plus			
$\frac{\text{Nitrogen retained, grams}}{54} \times 1000$	= Intracellular H ₂ O grams	= Water balance grams	$= \frac{\text{Sodium + Potassium retained, mM}}{160} \times 1000$

TABLE II
Detailed results in terms of 24 hours

Subject	Observation number	Days	Room		Intake										Total (calories)	Balance												
			Temperature	Relative humidity	Fat	CHO	N	Cl	Na	K	Ca	P	H ₂ O*	Fat		CHO	N	Cl	Na	K	Ca	P	H ₂ O†	Total weight				
			F	per cent	grams	grams	grams	mm.	mm.	mm.	mm.	mm.	mm.	grams	grams	grams	grams	mm.	mm.	mm.	mm.	mm.	mm.	grams	grams			
N. O.	1	6	77	50	12.1	23.8	0.84	4.5	8.5	8.0	3.9	3.0	333	114	6.8	0.0	0.41	1.1	1.5	1.9	0.74	1.4	17	23				
N. O.	2	6	77	50	12.3	24.3	1.44	8.5	4.6	8.4	6.6	6.1	293	135	6.3	0.0	0.64	2.0	2.3	1.7	2.4	2.1	23	33				
E. M.	3	11	77	50	12.0	24.8	1.88	8.0	4.9	11.3	9.1	8.3	297	163	1.6	0.0	0.66	1.3	8.0	1.3	1.7	3.1	24	33				
L. O.	4	6	77	40	9.5	41.3	1.78	6.8	4.9	9.5	8.9	7.5	431	183	7.5	4.8	0.58	1.9	1.6	3.5	5.6	5.3	31	40				
L. O.	5	8	77	40	10.3	44.3	1.98	7.1	8.0	8.9	9.4	7.9	462	188	8.0	5.1	0.94	4.4	4.9	7.5	3.7	33	45					
L. O.	6	4	85, 73	40	12.4	33.3	3.35	8.5	6.0	12.0	11.3	9.5	431	270	8.4	0.0	0.82	0.7	1.3	1.9	9.7	8.4	16	35				
L. O.	7	6	77, 72	40	15.4	68.5	3.78	10.6	7.4	14.5	14.0	11.5	843	337	11.6	0.3	1.07	1.4	1.3	3.3	11.9	8.3	13	40				
H. L.	8	7	72	40	12.8	28.3	2.49	9.5	6.7	12.3	12.5	10.8	319	199	8.1	0.0	0.83	1.5	2.3	2.1	7.9	3.3	13	25				

* Includes water of oxidation (2)

† Total calories were predicted from insensible perspiration in Observations 1 2 3 and estimated from observations in calculation of 2 to 5 hours combined with 24-hour minute to minute records of activity in remaining observations

‡ Direct organic method (2)

§ Perspiring profusely

|| Filter paper test (11) positive.

¶ Urinary calcium not determined

ganic method (2) the water intake consists of water ingested plus water of the food plus water of oxidation the latter derived from knowledge of the metabolic mixture. The water output consists of water excreted through the urine, feces, and skin and lungs, the latter derived from the total insensible weight loss and the metabolic mixture. According to the indirect organic method, the water balance is calculated by subtracting from the total weight change the weight of retained solids of which the organic solids, protein and fat, constitute the chief items. Although the indirect method uses the same fundamental assumptions as the direct in deriving the composition of the metabolic mixture, it involves the additional determination of dietary and fecal fat, and fecal nitrogen. The calculation is simpler than in the direct method.

Two methods of constructing the water balance from electrolyte balance were used, one from the chloride and nitrogen balance (1 8d) and the other from the sodium and potassium balance (7) In the anion method, the total water was partitioned into extracellular and intracellular phases on the basis of chloride and nitrogen retention. It was here assumed that chloride was retained extracellularly and nitrogen intracellularly in unit form concentrations of 120 mm. (8d) and 54 grams⁴ per liter of water respectively The use of nitrogen (1) as a measure of intracellular water accretion seemed particularly desirable because the uniformity in nitrogen retention from period to period fitted with the concept that these rapidly growing premature infants were adding protoplasm at a regular rate. In the cation method it was assumed that no changes in body concentration

of base took place from the beginning to the end of an observation and that the total water balance therefore equalled the sum of sodium and potassium retained divided by the concentration of total base in intra and extracellular water namely 160 mm. per liter (16) No analyses of serum were made.

RESULTS

The detailed results of the observations are presented in Table II, and a summary of the water balances in Table III Comparison of the results of direct and indirect organic methods (Columns 1 and 2 Table III) shows that in six

TABLE III

Summary of results of water balance calculated according to both organic and electrolyte methods

Subject	Observation number	Days	Organic method		Electrolyte method						Average organic	Average electrolyte
			Direct	Indirect	Anion				Cation			
					Extracellular (from Cl)	Intracellular (from N)	Total (2+4)	From Na and K	Total			
1	2	3	4	5	6	7	8					
			grams per 24 hours	grams per 24 hours	grams per 24 hours	grams per 24 hours	grams per 24 hours	grams per 24 hours	grams per 24 hours	grams per 24 hours		
N. O.	1	6	17	18	0	6	17	17	17	17		
N. O.	2	6	25	27	16	12	28	34	28	25		
E. M.	3	11	24	26	11	13	24	37	25	26		
L. O.	4	6	31	31	15	17	32	32	31	27		
L. O.	5	8	22	26	37	15	52	58	35	37		
L. O.	6	4	14	11	6	16	22	22	18	16		
L. O.	7	6	12	22	11	20	31	23	17	30		
H. L.	8	7	13	11	15	16	31	29	13	25		

⁴ This represents a recently determined concentration of nitrogen in the intracellular water of human muscle (15)

of eight observations (Observations 1, 2, 3, 4, 5, and 8) the water balances differ by 3 grams or less per 24 hours. Comparison of the results of the two electrolyte methods (Columns 5 and 6) shows that in all but two of the observations (Observations 6 and 8) the difference between the water balances is 4 grams or less per 24 hours. This agreement of results within each pair of methods serves as a check on the accuracy of the different analytical procedures used, although it is no measure of the validity of the assumptions underlying each pair of methods. The same assumptions (17) concerning the reliability of the respiratory quotient as an index of intermediary metabolism are used for both organic methods, and assumptions concerning the uniformity of concentration of electrolytes throughout various body fluids are used in both electrolyte methods.

To test the validity of both sets of assumptions we have in Table III, Columns 7 and 8, compared the average results of the organic methods with the average results of the electrolyte methods. It is seen that in 5 of the 8 observations (1, 2, 3, 4, and 6) the difference between results was 2 grams or less per 24 hours. In the remaining three observations (5, 7, and 8) the water balance as calculated from the electrolyte retention was from 13 to 32 grams too high. Appreciable excretion of chlorides through the skin was present in all three observations in which disagreement of results was found, suggesting that falsely high retentions of electrolytes were credited to the infants because no measure of skin excretion had been made (18).

To determine whether the agreement between results of the organic and electrolyte methods represented a valid support of the two methods or merely a fortuitous coincidence, we have in Table IV related the water balance to the body weight change. These infants were all receiving diets adequate to produce a satisfactory gain in weight. Under these conditions the percentage of weight gain consisting of water might be reasonably expected to approximate 60 to 80 per cent, since water comprises 70 per cent of infantile tissue by actual analysis. It is seen that, according to the direct organic method in six of eight observations, water comprised 51 to 75 per cent of the weight gain and averaged 61 per cent for the whole group. According to the indirect organic

TABLE IV
Relation of water balance to weight gain

Subject	Observation number	Weight gain	Amount of body weight as water				Remarks
			Organic method		Electrolyte method		
			Direct	Indirect	Anion	Cation	
		Grams per 24 hours	per cent	per cent	per cent	per cent	
N O	1	25	68	64	68	68	
N O	2	38	74	71	74	63	
E M	3	32	75	81	75	84	
L O	4	49	63	63	67	67	
L O	5	45	51	58	122	129	Perspiring profusely. Room temperature 87° F
L O	6	35	40	60	63	46	
L O	7	40	30	55	78	70	Filter paper test positive
H L	8	25	52	44	124	156	Filter paper test positive
Average (weighted)			61	63	72*	70*	

* Observations 5 and 8 omitted from these averages

method, water comprised 55 to 81 per cent of the weight gain in seven of eight observations and averaged 63 per cent for the whole group. According to the anion method, water comprised 63 to 78 per cent of the weight gain in six observations, and according to the cation method 63 to 84 per cent in five of eight observations. In Observations 5 and 8, the water as predicted from both anions and cations represented 122 to 156 per cent of the weight gain, impossible results which were undoubtedly due to the unmeasured excretion of electrolytes through the skin.

The evidence suggests that under normal conditions of weight gain, either method of assessing water balance gives results consistent with the concept that infantile tissue is 70 per cent water, but that in the presence of perspiration the excretion of electrolytes through the skin gives falsely high values for retention and therefore interferes with an accurate prediction of water balance.

In Table V is presented a comparison of the found and "theoretical" ⁵ retention of sodium,

⁵ The "theoretical" sodium retention was calculated from the chloride on the assumption that for every 120 mM. of chloride retained 148 mM. of sodium (8d) were retained, these being the approximate concentrations per liter of extracellular water. The "theoretical" potassium was calculated from the nitrogen retention by as-

TABLE V

Comparison of found and theoretical retentions of sodium potassium and phosphorus*

Subject	Observation number	Sodium		Potassium		Phosphorus	
		Found	"Theoretical"†	Found	"Theoretical"‡	Found	"Theoretical"§
N O	1	1.5	1.4	1.2	1.3	1.4	1.2
N O	2	2.2	2.5	1.7	2.0	2.1	2.7
E. M	3	3.0	1.6	1.3	2.0	2.1	2.0
L. O	4	1.8	2.4	3.3	2.7	5.3	5.7
L. O	6	1.3	9	1.3	2.5	6.4	7.5
Average (weighted)		2.1	1.7	1.7	2.1	3.2	3.5

* Observations 5 7 and 8 omitted because of perceptible perspiration

† Na mM. = $\frac{148}{120}$ Cl mM

‡ K mM = 3.1 N grams.

§ P mM = 0.6 Ca mM + 2 N grams.

potassium and phosphorus. Although they do not coincide in individual observations the weighted averages show a fair agreement for the 33 days of observation in which three infants gained a total of 1164 grams. The found and "theoretical" sodium retentions were 2.1 and 1.7 mM, the found and "theoretical" potassium retentions were 1.7 and 2.1 mM, and the found and "theoretical" phosphorus retentions were 3.2 and 3.5 mM per 24 hours, respectively. If allowance be made for excess sodium (8d) present in bone in a concentration of 1 mM of sodium for every 30 mM of calcium (20), the agreement between found (2.1 mM) and "theoretical" (1.9 mM) is closer.

The evidence suggests that over long periods of observation electrolytes are probably stored in the growing infant in concentrations approximating those demonstrated by tissue analyses. More exact definition of the range within which they vary may depend on further analyses of human tissues and carefully controlled balance studies.

assuming that for each gram of nitrogen retained, 3.1 mM of potassium were retained this being the approximate K/N ratio of human and dog muscle (8d, 16). The "theoretical" phosphorus retention was calculated from the calcium and nitrogen retention using a formula derived from the P/Ca ratio of bone (19) and the P/N ratio of muscle (8d, 16).

DISCUSSION

The two chief methods used for determining the total water exchange of these thriving premature infants are based on entirely different principles. In the organic method, the water balance is determined in part on the basis of the relation of water exchange to the metabolism of organic substances, protein, fat, and carbohydrate, accepting the respiratory quotient and the urinary nitrogen as indices of the composition of the metabolic mixture. In the second method water exchange is related to the metabolism of inorganic substances. Because of the fundamental needs for maintaining proper relations in the body between electrolytes and water (21), one might *a priori* consider a method of measuring water balance based on electrolytes as more desirable than one based on assumptions concerning organic metabolism in the use of which increasing caution is being advised (17). In practice, however, there is great difficulty in measuring electrolyte balance accurately, both because of the small absolute amounts normally retained and because unmeasured skin excretion results in crediting the subjects with false retentions of electrolytes and therefore of water. Under such conditions, the use of the method based on calculation of the composition of the metabolic mixture gives a more accurate measure of the water balance, provided the total 24-hour caloric expenditure has been reasonably approximated. The chief reason for this as the method applies to infants has already been pointed out (6b), namely, that water comprises by far the largest part of the intake urine, feces, insensible weight loss, and of shifts in body weight so that by accurate weighing of the subjects, his actual intake, and carefully collected samples of urine and feces, one directly arrives at an approximation of the water intake partition of output between urine, feces, and skin and lungs and the water balance.

It should be noted however that if special precautions are taken to prevent appreciable excretion of electrolytes through the skin by lowering room temperature, shedding clothes or limiting activity methods based on electrolyte exchange will give data not only concerning total water exchange but also on its partition into intracellular and extracellular compartments.

The agreement between the average found and "theoretic" balances of sodium, potassium, and phosphorus and the fact that the water balance as predicted from electrolyte balances represented approximately 70 per cent of the body weight gain demonstrate that in the tissue accretion of premature infants, just as in the loss of tissue by older children (1), electrolytes are added to the body in approximately the proportions normally present in body fluids

SUMMARY AND CONCLUSIONS

Eight observations of water and electrolyte balance, totalling 48 days, were concurrently made on four premature male infants on diets adequate to produce weight gains of 25 to 49 grams daily. The water balance was calculated from both the metabolic mixture, *tc*, from an estimate of the protein, fat, and carbohydrate oxidized, and from the electrolyte exchange. The close agreement between the results of both methods in five of eight observations suggests that under carefully controlled environmental and dietary conditions, either method of predicting the water balance of thriving premature infants is satisfactory. In two of three observations in which perceptible perspiration was present, the unmeasured excretion of electrolytes through the skin presumably resulted in crediting the subjects with a falsely high retention of electrolytes and water. Under such conditions the organic method yielded better estimates of water balance.

The similarity between the actual retentions of sodium, potassium, and phosphorus and "theoretic" retentions calculated from the chloride, nitrogen, and calcium and nitrogen retentions, respectively, indicate that in normal growth electrolytes are retained in approximately the relations to each other that exist in body tissues.

APPENDIX

Protocols

1 *Infant N O*, white, aged 17 days, weight 1912 grams at the onset of observation, was studied in two observations (Numbers 1 and 2) of six days each. He gained an average of 25 and 38 grams daily in these two observations. The room temperature was 77° F and the relative humidity 50 per cent in the two observations.

In Observation 1, he received human milk for

three days and a diet of evaporated milk, water, dextrimaltose, and olive oil of similar organic and fluid content for the succeeding three days. In Observation 2, he received a formula of human milk, casein, and dextrimaltose for three days, followed by a formula of evaporated milk, water, and dextrimaltose of similar organic and fluid content.

2 *Infant E M*, negro, aged 25 days, weight 2083 grams at the onset of Observation 3, was studied for eleven consecutive days. He gained an average of 32 grams per day during this observation. The room temperature was 77° F and the relative humidity 50 per cent during the observation.

During the first three days he received a formula of human milk, dextrimaltose, and calcium paracaseinate (casec), during the following eight days, a diet of evaporated milk, dextrimaltose, and water of similar organic and fluid content.

3 *Infant L O*, negro, aged 31 days, weight 2365 grams at the onset of observation, was studied in four observations under varying environmental conditions. In all observations he received a diet of a powdered skim-milk-olive oil preparation (olac), reinforced with dextrimaltose and water. His daily weight gain in the four observations averaged 49, 45, 35, and 40 grams respectively.

The first observation (Observation 4) consisted of three two-day periods at a temperature of 77° F and 40 per cent relative humidity. Between the second and third period of this observation an interval of three days elapsed during which he was exposed to a temperature of 87° F, with a relative humidity of 40 per cent for two days and 80 per cent for the final day. These three days in which visible perspiration was present constitute Observation 5.

In Observation 6, the subject, now 51 days of age and weighing 3197 grams, was studied for two two-day periods at temperatures of 72 and 68° F, respectively, with the relative humidity 40 per cent.

In Observation 7, *L O*, now 65 days of age and weighing 3795 grams, was studied for two days at a temperature of 77° F and for three days at a temperature of 72° F with the relative humidity 40 per cent throughout. In both periods the elimination of water through the skin and lungs was

considerably higher than in Observations 4 and 6 at similar temperatures and chlorides were excreted through the skin as indicated by the filter paper test.

4 Infant H L colored, aged 23 days and weighing 2564 grams at the onset of observation, was studied in a single observation of seven days, at an environmental temperature of 72° F and relative humidity of 40 per cent. A high insensible perspiration combined with a positive filter paper test, again indicated appreciable excretion of chlorides through the skin. His diet consisted of the powdered skim milk-olive oil preparation and water in amounts adequate to permit an average daily gain of 25 grams.

Chemical methods

The following methods were employed in triplicate for analysis of the diet, urine, and feces. The latter were dried by evaporation on a steam bath for 48 to 96 hours. Aliquots of the dried stool were used for fat determination and the fat was extracted from the remaining dried feces with a mixture of petroleum and ethyl ether. The remaining analyses were made on the result ing fat-free dried residue.

Water in urine and milk was determined by drying at 100° C for 48 hours, nitrogen by the Kjeldahl method, fat by the Roese Gottlieb method (22), and lactose in human and liquid cow's milk by a gravimetric method (23) for the dried cow's milk preparation, the carbohydrate content as submitted by the manufacturer was accepted.

Chlorides were determined by a modified Volhard titration method (24) phosphorus by the Tisdall method (25) and calcium by the McCrudden method (26). Samples were ashed at 500° C in a muffle furnace prior to determination of sodium by the Butler Tuthill (27) modification of the Barber Kolthoff method and potassium by the chloroplatinate method of Shohl and Bennett (28).

BIBLIOGRAPHY

- Gamble, J. L., Ross, G. S., and Tisdall, F. F., The metabolism of fixed base during fasting. *J Biol Chem.*, 1923 57 633
- Newburgh, L. H., Johnston, M. W., and Falcon Leases M., Measurement of total water exchange. *J Clin. Invest.*, 1929-30 8, 161
- Laviates P. H., The metabolic measurement of the water exchange. *J Clin. Invest.*, 1935 14 57
- Peters, J. P., Body Water Chapter VII. Charles C. Thomas Springfield, 1935
- Newburgh L. H., Johnston M. W., Lashmet, F. H. and Sheldon J. M., Further experiences with the measurement of heat production from insensible loss of weight. *J Nutrition* 1937 13, 203.
- (a) Levine, S. Z., and Wheatley M. A., Respiratory metabolism in infancy and in childhood. XVII. The daily heat production of infants—predictions based on the insensible loss of weight compared with direct measurements. *Am. J. Dis. Child.* 1936, 51, 1300
(b) Levine, S. Z., Wheatley M. A., McEachern T. H., Gordon H. H., and Marples E., XXI. Daily water exchange of normal infants. *Am. J. Dis. Child.* 1938 56, 83
- Laviates P. H., Desopo L. M., and Harrison H. E., The water and base balance of the body. *J Clin. Invest.* 1935 14 251
- (a) Darrow D. C., and Yarnet H., The changes in the distribution of body water accompanying increase and decrease in extracellular electrolyte. *J Clin. Invest.*, 1935 14 266
(b) Darrow D. C., and Yarnet, H., Metabolic studies of the changes in body electrolyte and distribution of body water induced experimentally by deficit of extracellular electrolyte. *J Clin. Invest.*, 1936 15 419
(c) Yarnet, H., Darrow D. C., and Carey M. K., The effect of changes in the concentration of plasma electrolytes on the concentration of electrolytes in the red blood cells of dogs, monkeys and rabbits. *J Biol Chem.* 1936, 112, 477
(d) Harrison, H. E., Darrow D. C. and Yarnet, H., The total electrolyte content of animals and its probable relation to the distribution of body water. *J Biol. Chem.*, 1936 113 515
(e) Harrison, H. E., and Darrow D. C., The distribution of body water and electrolytes in adrenal insufficiency. *J Clin. Invest.*, 1938 17 77
(f) Yarnet, H., and Darrow D. C., The effect of hyperthermia on the distribution of water and electrolytes in brain, muscle and liver. *J Clin. Invest.*, 1938, 17 87
(g) Yarnet, H., and Darrow D. C., The effect of growth on the distribution of water and electrolytes in brain, liver and muscle. *J Biol Chem.*, 1938 123, 295
- (a) Hastings A. B., and Eichelberger L., The exchange of salt and water between muscle and blood. I. The effect of an increase in total body water produced by the intravenous injection of isotonic salt solutions. *J Biol. Chem.*, 1937 117 73
(b) Eichelberger L. and Hastings A. B., II. The effect of respiratory alkalosis and acidosis induced by overbreathing and rebreathing. *J Biol Chem.*, 1937 118, 197

- (c) III The effect of dehydration. *J Biol Chem*, 1937, 118, 205
- (d) Manery, J F, Danielson, I S, and Hastings, A B, Connective tissue electrolytes *J Biol Chem*, 1938, 124, 359
- 10 Levine, S Z, Wilson, J R., and Kelly, M, The insensible perspiration in infancy and in childhood I Its constancy in infants under standard conditions and the effect of various physiologic factors *Am. J Dis Child*, 1929, 37, 791
- 11 Vasti, A, The insensible water loss through the skin *Am. J Physiol*, 1932, 102, 60
- 12 Gordon, H H, and Levine, S Z, Respiratory metabolism in infancy and in childhood. XVIII The respiratory exchange in premature infants Basal metabolism. *Am. J Dis Child*, 1936, 52, 810
- 13 Gordon, H H, and Kelly, M D, XIX The respiratory exchange in premature infants—elimination of water through the skin and the respiratory passages *Am. J Dis Child*, 1936, 52, 1100
- 14 Levine, S Z, McEachern, T H, Wheatley, M A, Marples, E., and Kelly, M D Respiratory metabolism in infancy and in childhood XV Daily energy requirements of normal infants *Am. J Dis Child*, 1935, 50, 596
- 15 Harrison, H E., Personal communication
- 16 Peters, J P, and Van Slyke, D D, Quantitative Clinical Chemistry Vol I Interpretations Williams and Wilkins Co, Baltimore, 1931, p 753
- 17 Mitchell, H H., Some problems in the study of energy metabolism. Report of the conference on energy metabolism, June 1935 Under the auspices of the Committee on Animal Nutrition, National Research Council, Washington, D C
- 18 (a) Romunger, E, and Meyer, H., Über die Mineralausscheidung durch die Haut beim Säugling *Ztschr f Kinderh*, 1929, 47, 721
- (b) Swanson, W W, and Iob, L V, Loss of minerals through the skin of infants *Am. J Dis Child*, 1933, 45, 1036
- (c) Wheatley, M A., and Levine, S Z, Mineral balance in normal infants *Am J Dis Child (Proc.)*, 1934, 48, 1432.
- (d) Freyberg, R. H, and Grant, R. L., Loss of minerals through the skin of normal humans when sweating is avoided *J Clin Invest.*, 1937, 16, 729
- 19 Albright, F, Bauer, W, Ropes, M, and Aub, J C, Studies of calcium and phosphorus metabolism. IV The effect of the parathyroid hormone. *J Clin Invest.*, 1929, 7, 139
- 20 Harrison, H E., The sodium content of bone and other calcified material *J Biol Chem*, 1937, 120, 457
- 21 Henderson, L J The theory of neutrality regulation in the animal organism. *Am J Physiol*, 1908, 21, 427
- 22 Roes-Gottlieb method, Association of Official Agricultural Chemists Official and tentative methods of analysis compiled by the Committee on Editing Methods of Analysis, Washington, D C, Association of Official Agricultural Chemists, 1930, page 217
- 23 Lactose—Official gravimetric method of the Association of Agricultural Chemists *Ibid*, page 216
- 24 Peters, J P, and Van Slyke, D D, Quantitative Clinical Chemistry Vol II Methods Williams and Wilkins Co, Baltimore, 1932, p 833
- 25 Tisdall, F F, A rapid colorimetric method for the quantitative determination of the inorganic phosphorus in small amounts of serum *J Biol Chem*, 1922, 50, 329
- 26 McCrudden, F H, The determination of calcium in the presence of magnesium and phosphates The determination of calcium in urine *J Biol Chem*, 1911-12, 10, 187
- 27 Butler, A M, and Tuthill, E, An application of the uranyl zinc acetate method for determination of sodium in biological material. *J Biol Chem*, 1931, 93, 171
- 28 Shohl, A T, and Bennett, H B, A micro method for the determination of potassium as iodoplatinate. *J Biol Chem*, 1928, 78, 643

EXPERIMENTAL HYPOSTHENURIA¹

By J. M. HAYMAN JR., N. P. SHUMWAY, P. DUMKE, AND MAX MILLER²

(From the Department of Medicine Western Reserve University Medical School and the Lakeside Hospital Cleveland)

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The clinical usefulness of the specific gravity test of kidney function and the variety of conditions under which impairment of concentrating ability is encountered, furnished the incentive for this study. This test is most commonly used as an indication of the degree of renal damage in glomerulonephritis and arteriolar nephrosclerosis in both of which there is a significant reduction in the number of nephrons. A urine of low specific gravity however, is also encountered in some cases of acute nephritis, many acute infections, chemical poisoning, prostatic obstruction, pyelonephritis, trauma to the kidney and severe anemia in which there is usually no significant reduction in the number of nephrons. It seemed proper, therefore, to attempt to determine whether loss of concentrating power was due to a single mechanism, or whether it might be brought about in more than one way.

The clinical importance of loss of concentrating power has been recognized since the papers of Blackall (12) in 1820 and Bright (14) in 1827 and interest in the mechanism producing it evidenced by the many explanations that have been offered (73). Christison in 1839 (21) was apparently the first to employ a concentration test. He determined the specific gravity of morning urine, giving the average normal as 1.024 or 1.025 with a range from 1.016 to 1.030 while in patients with "granular kidneys" it fell below 1.016. He cautions that the gravity should be corrected for any protein present.

Christison (21) and Rayer (66) interpreted the polyuria as a compensatory mechanism for the loss of ability to concentrate solutes. Bartels (9) accepted Traube's hypothesis that destruction of renal mass led to an ele-

vated blood pressure and cardiac enlargement, so that more blood was forced "through the urinary apparatus" and noted that when the heart failed, "the abnormally large amount of urine falls off and the abnormally low specific gravity rises." Johnson (41) believed the polyuria unrelated to the arterial tension, but caused by the diuretic influence of some abnormal products in the circulation. Newman (61) suggested that the polyuria of the contracted kidney was due to obstruction of the lymphatics. Thoma (82) thought it due to increased glomerular permeability. v. Kórányi (91) and his associates who investigated hyposthenuria extensively offered only the suggestion that with failing kidney function the capacity of the kidney to do the work entailed in the processes of concentrating or diluting solutes withdrawn from the blood progressively diminishes. Schlager, Hedinger and Takayasu (74) believed the polyuria of Bright's disease to be due to hyperirritability of damaged renal vessels in response to a diuretic stimulus.

Muller (60) did not regard hyposthenuria as the necessary result of reduction in kidney mass. He could not see why a small mass could not put out a urine of normal specific gravity. He believed both the polyuria and hyposthenuria of infectious disease, urinary obstruction, glomerulonephritis and vascular disease to be due to the vicarious secretion of water by the tubules.

Vollhard (90) emphasized the wide variety of conditions in which loss of concentrating power occurs. In those in which kidney mass is reduced the remaining nephrons respond by a "compensatory" polyuria, as does the normal kidney to increased demand for elimination of waste. This polyuria exhausts the secretory apparatus (granules and vacuoles) in the tubule cells so that excretion of a concentrated urine is impossible. When there is no reduction in kidney mass he believed the tubule secretory apparatus is primarily damaged by the poison or by increased pressure in the peritubular capillaries.

Mayra (55) believed that while the rapid passage of fluid down the remaining tubules may contribute to the polyuria of chronic nephritis, the chief fault must be in the inability of the diseased tubule cells to overcome as great an osmotic pressure as in health.

Fremont Smith *et al.* (27) suggested that a large volume of urine is derived from a small number of glomeruli with all their capillaries open, a small volume of concentrated urine from a larger number of glomeruli with only a few capillaries open in each. This is contrary to Verne's (89) hypothesis based on his experi-

¹ The results of some of these experiments were presented before the Fifty First Annual Meeting of the Association of American Physicians, May 6 1936 (Tr. A. Am. Physicians, 1936, 51 453).

² The expenses of this investigation were defrayed in part by a grant from the Commonwealth Fund.

³ Dr. Shumway took part in the earlier experiments while serving as Assistant Resident. Dr. Dumke carried out most of the experiments on ureteral obstruction during his fourth year in medical school. Dr. Miller joined in the later experiments.

Rehberg (68) pictured the mechanism bringing on polyuria as follows "When the filtrate rate is considerably decreased, nitrogen retention in the blood begins. The result is that the glomerular filtrate contains a much higher concentration of nitrogenous substances than usual, so that even with normal tubules the concentration limiting the reabsorption of water is reached at an earlier stage. Consequently, a larger amount of fluid is left which cannot be reabsorbed, a condition even more pronounced if the tubules are injured also" Hyposthenuria and isosthenuria would be explained in the same way.

Govaerts's (32) only suggestion was that in terminal nephritis the number of glomeruli may be so reduced that the volume of glomerular filtrate cannot allow for any variation in water output.

Fishberg (25) emphasized "the unitary nature" of impairment of renal function, that "in almost all diseases which cause widespread injury to the kidney there is loss of concentrating ability, which applies to each and every urinary constituent." He believed loss of concentrating ability is almost always associated with a diminution in the number of functioning renal units and increase in the amount of filtrate per unit. Akerren (3) offered the same explanation for the function of the Schrumpfniere. He believed the histological changes seen in the tubule cells did not necessarily have anything

do with the increase in urine volume or loss of concentrating power. This hypothesis (of Fishberg and of Åkerrén) will not account for hyposthenuria with a normal number of nephrons.

Chasis and Smith (19) suggested, from studies of inulin/urea clearance ratio, that failure of the "facultative" reabsorption of water in the distal tubule leads to the clinical condition of polyuria and hyposthenuria, while impairment of the "obligatory" reabsorption in the proximal tubule, perhaps brought about by excessive excretion of base and chloride, leads to isosthenuria.

It is apparent from this review that while some authors have been impressed by the variety of clinical and pathological conditions accompanied by hyposthenuria, most have attempted to explain it by a single mechanism in all cases, either tubular damage or the rapid passage of an abnormally large quantity of fluid down a small number of tubules.

In order to investigate whether a single mechanism is adequate to explain all cases it seemed appropriate to produce polyuria and hyposthenuria in dogs by various experimental means and then to study the ability of these animals to excrete a concentrated urine under various circumstances. If any conditions could be found which caused excretion of a urine of high specific gravity, it seemed reasonable to assume that in such cases the tubular cells were still normal (or else that

the circumstances of the experiment had led to their recovery) and that the mechanism of the hyposthenuria did not lie primarily in parenchymal damage.

METHODS

The experimental methods used to produce hyposthenuria were reduction in kidney mass, uranium poisoning, and ureteral obstruction. In addition, some observations have been made on the effect of denervation, diet, anemia, vitamin B₁₂ deficiency, pregnancy, and constriction of the renal arteries on concentrating ability.

All experiments were made on healthy female mongrel dogs of unknown age, weighing from 5 to 25 kgm. They were kept in well ventilated cages and fed a stock diet of Ralston's Purina Dog Chow. All were observed at least four weeks before being used.

When hyposthenuria had been produced, attempts to obtain a concentrated urine fell into several groups.

1 *Diet* Concentrating ability on low and high protein diets were observed in normal dogs and after subtotal nephrectomy. Jolliffe and Smith (42) showed that creatinine and urea clearances were reduced on a low protein diet, but did not study concentrating ability. Their original cracker meal diet was used for low protein periods in some experiments, in others Pitts' modification (64) was used. One pound of ground lean meat, alone or plus 5 grams NaCl daily, furnished the high protein diets. One animal was given Whipple and Robscheit-Robbins' (92) bread diet and salmon.

2 *Hormones* Pituitrin in doses of 40 to 80 international units was given over a period of 2 to 8 hours after water had been withheld for 24 hours and the highest specific gravity obtained on several catheter specimens during a 12-hour period recorded. The effect of adrenal cortical extract (eschatin, 2 to 10 cc.) was studied in a like manner.

3 *Increased concentration of salts in the glomerular filtrate* Sodium sulphate, or a mixture of sodium sulphate and bisodium phosphate, in doses of 0.5 to 1.0 gram per kgm was injected slowly, intravenously, at the end of a 24-hour concentration test and the maximum specific gravity obtained during the following 7 to 12 hours recorded. These salts were used since Alving and Van Slyke (5), and Addis and Foster (2) have called attention to the fact that sulphates and phosphates have a greater effect on urinary specific gravity, for a given concentration, than any of the other salts or urea.

4 *Attempts to increase the plasma colloid osmotic pressure, and so reduce the effective filtration pressure in the glomerular capillaries* The means used were intravenous injections of acacia, dehydration by croton oil (or magnesium sulphate) and arica nut catharsis, injection of dog plasma concentrated by freezing and drying, or by Thalhimer's (80) method of evaporation in cellophane tubing, or by intravenous injections of sucrose. When sucrose was used, 35 to 50 cc. of a 50 per cent solution was given in the morning and the dog put in a metabolism cage. A second dose was usually

given in the afternoon. The next morning the animal was catheterized, and this added to the cage specimen. These specimens all contained large amounts of sucrose, the specific gravity of course depending on the total volume. The animal was catheterized again after 2 to 3 hours. This specimen usually had less than 1 per cent sucrose and the gravity given is corrected for sucrose. This was done because our interest is in the ability of the kidney to concentrate normal urinary constituents and we have no data on the ability of the diseased human kidney to concentrate sucrose under similar conditions for comparison.

5 *Decreased rate of filtration in order to allow more time for reabsorption in the tubule* Under sodium pentobarbital anesthesia, sufficient spinocaine was injected subdurally (after laminectomy) to lower blood pressure to 80 to 100 mm. Hg recorded from a cannula in carotid artery. In one animal (Dog 64) Dr Goldblatt put a clamp around the aorta above the renal arteries and constricted it sufficiently to lower femoral pressure to about 80 mm. Hg.

The ability of an animal to excrete a concentrated urine under control conditions and after injury was judged by a "concentration test." After trial of several techniques that adopted consisted of emptying the bladder by catheter, and then placing the animal in a metabolism cage without food or water. After 24 hours, the animal was catheterized again, cage and catheter specimens combined, and the volume and specific gravity corrected for any protein present, recorded. This technique naturally raises the question of the propriety of using the specific gravity of the whole 24-hour specimen. In most of the clinical concentration tests the specific gravity of the urine passed during the latter part of a period of dehydration is used as a measure of concentrating ability. It might seem that it would have been better to have followed a similar procedure with the dogs and recorded only the gravity of the urine passed during the latter part of a 24-hour period. The relative volumes of cage and catheter specimens varied tremendously frequently no cage specimen was obtained, the catheter specimen representing the entire 24-hour excretion. The gravity of urine passed during the latter part of a 24-hour period of water deprivation was not significantly higher than that passed during the earlier part with a sufficient frequency to justify a more elaborate technique. In 50 concentration tests the specific gravity of cage and catheter specimens were determined separately in 31 the catheter specimen was of higher specific gravity in 19 lower in 30 or 60 per cent of the determinations the specific gravity of the catheter specimen was within 0.005 of the cage specimen. The mean specific gravity of the catheter specimen exceeded that of the cage specimen by 0.0024 its standard deviation being 0.0071.

A single concentration test may at times fail to give a reliable estimate of an animal's ability to concentrate. That is, animals fed on the same diet and subjected to repeated concentration tests by the above technique will occasionally show gravities distinctly lower than the range on other tests. We have no satisfactory explanation

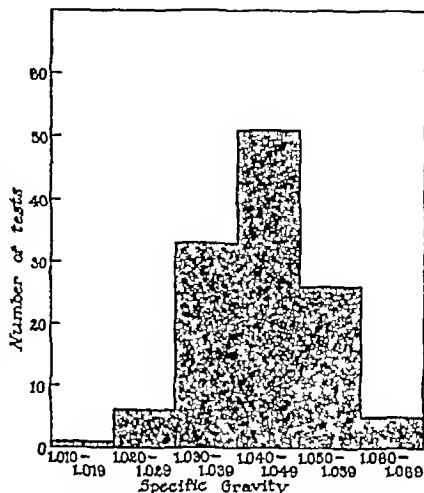


FIG. 1 DISTRIBUTION OF SPECIFIC GRAVITIES IN 122 TESTS ON 46 NORMAL FEMALE DOGS

tion for this and the possible contributing factors have not been investigated. Figure 1 shows the distribution of specific gravities in 122 tests on 46 normal female dogs. On repeated tests all could excrete urine of higher specific gravity than 1.030 41 or 89 per cent, better than 1.040 21 or 46 per cent, better than 1.050 and 5 or 11 per cent, better than 1.060. For this reason it has been necessary to carry out several tests on any animal both before and after any experimental procedure. While in general the urine volumes tended to be lower with higher specific gravities the correlation was poor. There was no relation between size of animal and maximum specific gravity over the size range used. Withholding water for 48 hours as a rule yielded a urine of somewhat higher gravity than that obtained after 24 hours. The average increase was 0.007 (Table I).

When urine volumes were adequate, specific gravities were determined to the fourth place by a Westphal balance with a 10 cc. plummet at 20° C. and compared to water at 4°. Where volumes were small, gravities were determined by pycnometer at room temperature and corrected to 20° assuming a linear relation between the specific gravity of urine and water over this small range of temperature differences. All gravities were corrected for protein, using Lashmet and Newburgh's factor (47). Proteins were determined by Shewky and Stafford's method (76) after calibration of the volume of precipitate per gram of protein by macro-Kjeldahl analyses. The specific gravity figures have been rounded off to three places to save space.

Creatinine and urea clearances were determined on the

TABLE I

Urine specific gravity for first and second 24 hours of a 48 hour concentration test in normal animals and after subtotal nephrectomy

Dog	Normal		Dog	Subtotal nephrectomy	
	First 24 hours	Second 24 hours		First 24 hours	Second 24 hours
33	1 044	1 055	3	1 028	1 025
36	1 042	1 052	4	1 020	1 023
37	1 034	1 037	8	1 016	1 021
38	1 042	1 045	15	1 029	1 037
39	1 050	1 064	17	1 010	1 012
40	1 051	1 044	19	1 025	1 022
	1 044	1 061	41	1 026	1 034
			44	1 023	1 023

majority of animals, inulin on a few Creatinine was estimated by Rehberg's (67) method. Two to three grams of creatinine were given by stomach tube one and one-half hours before the test. Usually blood samples were taken at the beginning and end of each collection period, occasionally in the middle of each period. The length of collection periods varied from 10 minutes to one hour, depending on the urine flow. The bladder was emptied by catheter at the end of each period. We have not obtained more complete emptying of the bladder by washing with saline or injection of air than by a properly done catheterization. The adequacy of this method has been shown (1) by recovering only insignificant amounts of the test substance in washings, and (2) by laparotomy after careful catheterization. Urea in urine was estimated by Van Slyke's (85) gasometric urease method, in blood by the same method in the earlier experiments, in the later ones by the hypobromite method (86). Inulin was administered intravenously in doses of 1 to 2 grams per kgm. and estimated in an iron filtrate (77) of plasma and urine by the Shaffer-Somogyi (75) method, before and after acid hydrolysis (72). Blood samples were centrifuged immediately after being drawn.

Blood pressure was determined by Jensen and Apfelbach's (39) direct method. Glomeruli were counted by a modification of Kunkel's (45) method, which permits a correction for uninjected glomeruli. It is assumed, for the purpose of estimating the original equipment of an animal, that the number of glomeruli in each of the kidneys was approximately equal.

Subtotal nephrectomy

Polyuria and hyposthenuria have been repeatedly produced experimentally by reduction in kidney mass. The literature is reviewed by Chanutin and Ferris (17). Reduction in functioning mass has been accomplished by surgical removal of renal tissue, ligation of branches of the renal arteries, injection of non-absorbable particles into the renal artery, and exposure of the kidney to

x-ray. The results have not been entirely consistent, due to the survival of varying amounts of kidney tissue and presumably to the various experimental methods used. Some of the earlier investigations were more concerned with the relation of the kidneys to metabolism and the existence of an internal secretion of the kidney than they were with functional disturbances.

The first partial nephrectomy was done in 1889 by Tuffier (84) who removed one kidney and then part of the other in dogs. He noted no change in the elimination of urine or urea, and that 1.5 grams of kidney per kgm was compatible with life. De Paoli (22) in similar experiments on cats, dogs, and rabbits believed one-half of one kidney the minimal amount for survival. Bradford (13) found that removal of approximately two-thirds of the renal tissue was followed by a marked and persistent polyuria, and that the greater the amount of tissue removed, the greater was the polyuria. There was an accompanying reduction in specific gravity from the normal of 1.030 to 1.050 to from 1.010 to 1.020. He states that the dogs were unable to concentrate their urine or to put out a high concentration of urea. Passler and Heinecke (62) noted the polyuria, but did not record specific gravities. Polyuria was also noted by Janeway (38), Allen, Scharf, and Lundin (4), Lundin and Mark (50), Hartman (33), Apfelbach and Jensen (7), and Chanutin and Ferris (17). No change in urine volume was found by Bainbridge and Beddard (8), Pilcher (63), Anderson (6), Mark (52), Mark and Geisendörfer (53), and Cash (15). A fixed low specific gravity, with inability to concentrate was recorded by Anderson, Mark, Lundin and Mark, Hartman, Apfelbach and Jensen, and Chanutin and Ferris, no change in specific gravity by Bainbridge and Beddard, Karsner, Bunker, and Grabfield (43), and by Cash.

Arterial hypertension also was recorded by Passler and Heinecke, Janeway, Allen, Scharf, and Lundin, Mark and Geisendörfer, Lundin and Mark, Hartman, Chanutin and Ferris, and Wood and Ethridge (93), only in the postoperative period by Cash and by Ferris and Hynes (24). No effect on blood pressure was found by Anderson, by Apfelbach and Jensen, and by Adams, Egloff, and O'Hare (1). The elevation of blood pressure in dogs, when present, was only slight or moderate, 10 to 35 mm Hg, and not of the same order as that obtained by Goldblatt *et al* (30) by constricting the renal arteries. Cash believed two factors necessary for the production of hypertension, reduction of renal tissue to 50 per cent of normal and the presence of necrotic renal tissue, a conclusion challenged by Chanutin and Ferris who found the hypertension to persist in rats after all necrotic tissue had been absorbed. By ligation of both poles of one kidney and removal of the other in rats, Chanutin and Ferris (17) produced a chronic renal insufficiency characterized by polyuria, low fixed specific gravity, albuminuria, nitrogen retention, hypertension, and

cardiac hypertrophy. The polyuria seen early without hypertension was thought to be due to increased glomerular permeability. When pathological changes in the renal rest had taken place, as indicated by albuminuria and elevation of the nonprotein nitrogen there was generally a hypertension. This was assumed to be a compensatory mechanism to maintain an increased volume of urine, and the polyuria was believed dependent to a great extent on the increased blood pressure. The pathological changes were presumably due to the protein in the diets (16) since it was more marked on high protein diets. Mark also produced a rapidly fatal insufficiency in subtotal nephrectomized dogs by feeding meat. Chanut and Ludewig (18) believed the urea clearance a good indicator of the degree of renal damage while the concentration test showed only qualitative reduction in function, since it might be low with normal clearances while reduced clearances were always accompanied by low

specific gravity. This conclusion is at variance with that of Alving and Van Slyke in man (5).

Since other factors such as necrotic tissue, fibrosis, possible tubular damage, and inflammation are present when functioning kidney mass is reduced by ligation of arteries radiation, or injection of foreign material, surgical removal was selected as the method best adapted to yield an uncomplicated picture of the effects of reduction in kidney mass.

After a preliminary period of observation, healthy female mongrel dogs were anesthetized with ether and approximately one third of the right kidney was removed through a lumbar incision. Two to six weeks later the left kidney

TABLE II
Summary of data before and after operation on animals subjected to subtotal nephrectomy†

Dog	Weight	Before operation						After operation						Kidney weight and glomerular count		Survival
		Concentration test		Mean clearance		Blood urea nitrogen	Mean blood pressure	Concentration test		Mean clearance		Blood urea nitrogen	Mean blood pressure	Left	Right	
		Volume, Mean, Range	Specific gravity, Mean, Range	Creatinine	Urea			Volume, Mean, Range	Specific gravity, Mean, Range	Creatinine	Urea					
	kgm.			cc. per min.	cc. per min.	mgm. per cent	mm. Hg			cc. per min.	cc. per min.	mgm. per cent	mm. Hg	grams, thousands	grams, thousands	months
3	12.5	152 83-270	1.038(3) 1.037-41				180(2)	273 160-418	1.023(7) 1.019-28			18.5	175(2)	28.0	23.5 36	1.6
4	10.5	95 43-155	1.038(3) 1.034-48				145(2)	169 110-244	1.021(4) 1.020-23				172(2)	28.0 214	15.5 60	3.5
6	8.0	91 90-92	1.040(2) 1.035-45	31.0	13.5	24.1	114(2)	204 144-275	1.017(5) 1.015-28			62.6	165(2)	28.0 346	12.0 98	2.8
8	13.3	195 81-242	1.030(4) 1.026-34	45.0	33.0	8.7	125	545 235-1016	1.015(4) 1.013-16	10.7	5.4	63.0	175(3)	43.5 395	16.3 86	1.0
9	17.6	177 100-235	1.034(3) 1.033-35	56.5	27.5	15.2	115	397 220-650	1.021(5) 1.015-25	20.7	9.1	32.4	153(3)	38.5 212	28.0 140	1.0
10	12.0	108 88-128	1.042(2) 1.037-46	46.5	27.2	24.0	114(5)	248	1.017	4.7	3.1	71.0	145	30.0 442	16.5 64	1.0
15	17.8	164 85-340	1.037(4) 1.025-44	62.7	32.7	11.0	125(2)	248 59-370	1.025(7) 1.018-29	34.4	16.2	35.5	142(2)	33.0 376	62.5 254	2.5
17	8.4	115 87-135	1.052(3) 1.040-66	51.9	13.6	13.4	130(2)	427 330-620	1.011(6) 1.009-14	5.4	3.0	107.0	140(8)	31.5 201.0(?)	14.8 62	5.0
19	10.7	141 56-267	1.040(5) 1.012-57	39.1	19.9	22.2	140(2)	252 223-290 416 222-730	1.021(8) 1.016-25 1.014(9)* 1.011-16	22.5	13.8	21.9	155(9)	77.5 556	30.0 68	27.5
41	11.0	111 39-140	1.038(5) 1.021-37	33.6 31.6	21.4	12.0	142(4)	86 62-130 183 130-225	1.036(2) 1.035-37 1.021(3) 1.016-27	41.5 39.0 16.2 15.6	20.0	13.2	168(9)	29.5 384	21.5 125	7.0
44	8.8	60 40-84	1.044(5) 1.036-48	29.6 29.7	13.1	14.4	128(3)	103 63-144 173 115-360	1.036(2) 1.032-39 1.017(5) 1.014-21	24.4 23.4 10.1 10.1	13.6	15.6	157(13)	22.0 394	17.3 100	7.0

† Figures in parentheses indicate the number of observations averaged.

* After March 1 1938

† Inulin clearance.

was removed. Observations were begun about a week after the second operation. In two animals (41, 44) concentration tests and clearances were determined between the first and second operations. The dogs were sacrificed after from 1 to 28 months. As is shown in Table II, and Figure 2, after operation the urine volume on concentration tests was increased and the specific gravity reduced. The specific gravity did not increase significantly after deprivation of water for 48 hours (Table I) except in two animals (Numbers 15 and 41) which had the greatest amount of remaining renal tissue. This is in sharp contrast to the moderate rise in normal animals for the last half of a 48-hour test. The creatinine and urea clearances were significantly reduced after operation. In Dogs 41 and 44, the inulin and creatinine clearances remained equal as kidney mass was reduced. None of the animals showed the marked rise in blood pressure which Goldblatt obtains by constricting the renal arteries. The rise in pressure varied from about 10 to 15 mm Hg in three animals to approximately 50 in two others. All of the animals showed some nitrogen

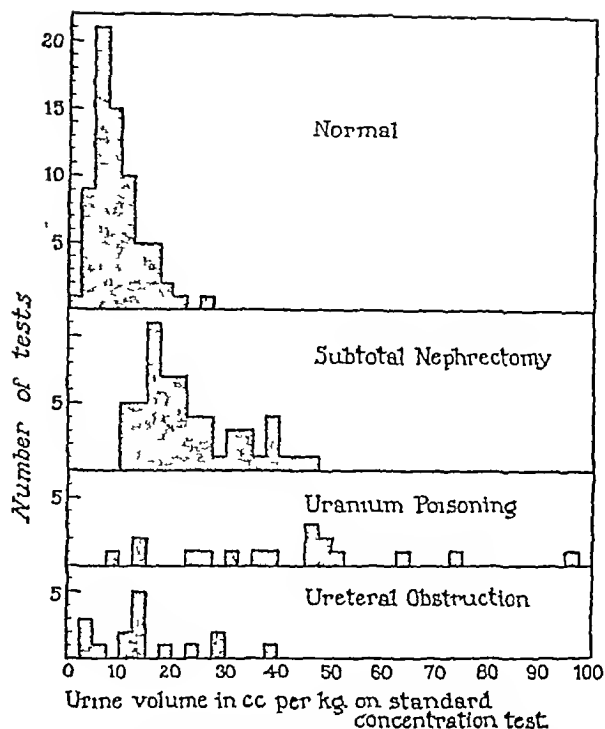


FIG 2 HISTOGRAM OF URINE VOLUMES ON CONCENTRATION TESTS IN NORMAL DOGS AND AFTER VARIOUS PROCEDURES

TABLE III

Effect of diets on kidney function tests in normal dogs and after subtotal nephrectomy

Dog	Stock diet				Cracker meal				Meat diet				Meat + NaCl			
	Specific gravity on concentration test	Mean clearance		Blood urea nitrogen	Specific gravity on concentration test	Mean clearance		Blood urea nitrogen	Specific gravity on concentration test	Mean clearance		Blood urea nitrogen	Specific gravity on concentration test	Mean clearance		Blood urea nitrogen
		Creatinine	Urea			Creatinine	Urea			Creatinine	Urea			Creatinine	Urea	
		cc. per min. ult	cc. per min. ult	mgm. per cent		cc. per min. ult	cc. per min. ult	mgm. per cent		cc. per min. ult	cc. per min. ult	mgm. per cent		cc. per min. ult	cc. per min. ult	mgm. per cent
NORMAL DOGS																
50	1 055	37 8	22 4	10 5	1 035	26 7	17 7	7 4	1 054	43 7	26 1	12 0	1 049	58 7	31 9	11 7
53	1 061	42 5	24 9	9 8	1 026	26 8	17 3	8 5	1 055	41 7	24 2	20 9	1 044	45 6	26 5	14 6
SUBTOTAL NEPHRECTOMIZED DOGS																
9	1 019	19 1	11 5	29 8	1 021	21 8	10.5	17 9	1 023	19 0	5 1	50 0	1 025	22 4	10 4	32 9
15	1 029	25 6	11 6	22 0	1 022	26 1	11 7	18 7	1 018	36 7	18 4	18 9	1 029	52 5	18 6	14 1
19	1 028	23 3	16 1	23 3	1 027	25 7	14 0	11 5	1 027	19 1	11 1	27 0	1 027	22 0	11 2	26 0

retention. All were in good condition when sacrificed except Dog 19. This animal remained in excellent condition from January, 1936, to August 1937. After this time, she began to lose weight and developed a progressive decrease in concentrating ability and clearances and increasing nitrogen retention, but no further elevation in blood pressure and no anemia.

The ability to excrete a dilute urine was preserved in the four animals in which it was tested (Dogs 3, 10, 41, 44) as shown by gravities of less than 1.002 after administration of water by stomach tube.

Table III shows the effect of low and high protein diets on function tests in two normal animals (Dogs 50 and 53) and in three after subtotal nephrectomy (Dogs 9, 15, and 19). In the normal animals the change in clearances is in the same direction, though less marked than those described by Jolliffe and Smith. There is also a failure of the normal dog to excrete a concentrated urine on the cracker meal diet. The partially nephrectomized dogs on the other hand show no consistent variation in clearances, and no variation in concentrating ability. In one animal (Dog 9) there is a hint that addition of salt to a high protein diet may have been followed by a decrease in blood urea nitrogen (28, 46).

Table IV shows the effect of pituitrin, eschatin, intravenous injection of hypertonic sulphate, increase in plasma colloid, and low blood pressure on urinary specific gravity. After pituitrin there was a slight increase in specific gravity in four of six animals above that of the maximum concentration test after operation. In no case did it reach the mean concentration test gravity before operation, and in only one instance did it exceed 0.002. In five of six normal dogs given large doses of pituitrin at the end of a concentration test, higher gravities were obtained than after any 24 hours without water but in only one animal did the difference exceed 0.009. In two of four of these dogs deprived of water for 48 hours higher gravities were obtained than after pituitrin, in one the gravity was 0.003 and in the other 0.011 lower than after pituitrin. This agrees with the well known fact that in normal dogs pituitrin diminishes urine volume and with this increases specific gravity, but as a rule it does not go significantly higher than after water deprivation alone. With

TABLE IV
Effect of various procedures on urinary specific gravity after subtotal nephrectomy, uranium poisoning and ureteral obstruction

No.	Specific gravity on test		After pituitrin	After eschatin	Increased plasma colloid			Low pressure	
	Before operation	After injury			Specific gravity	Change in plasma protein	Method	Specific gravity	Mean blood pressure
	Mean	Maximum							

SUBTOTAL NEPHRECTOMY

3	1.028	1.028	1.029	1.012				1.040	70
4	1.028	1.023	1.018	1.023	1.029		Ascorbic		
6	1.040	1.015		1.031	1.022		Ascorbic	1.036	57
8	1.030	1.018	1.018	1.025				1.027	74
9	1.034	1.021			1.041	5.5-9.8†	Croton oil Arice nut	1.033	58
10	1.012	1.016	1.018	1.028					
15	1.027	1.029	1.026*		1.028	5.0-6.7	Croton oil Arice nut		
19	1.040	1.037	1.018	1.024	1.045	5.5-7.3	Croton oil Arice nut	1.036	47
41	1.033	1.027			1.029	5.5-8.8	Saccharose	1.016	100
44	1.044	1.021	1.025		1.040	5.0-7.8	Concentrated plasma		
77	1.023	1.014	1.011*						

URANIUM POISONING

7	1.048	1.019	1.010	1.018				1.015	100
11	1.051	1.020	1.003						
12	1.041	1.009		1.018					
18	1.044	1.019		1.023					
14	1.038	1.017	1.021		1.013	7.8-8.2	Croton oil Arice nut	1.018	76
48	1.040	1.010		1.018					
46	1.035	1.021		1.023	1.022	8.0-7.1	Saccharose		
54	1.041	1.018			1.017	7.0-7.7	Saccharose	1.012	87

URETERAL OBSTRUCTION

20	1.043	1.018	1.017						
31	1.043	1.037	1.029	1.013	1.019	4.8-5.8†	Saccharose	1.018	65
23	1.033	1.015		1.018					
25	1.044	1.018		1.014					
29	1.032	1.023		1.031	1.018	6.5-8.0	Saccharose	1.012	75
31	1.034	1.018	1.030	1.013	1.018	7.0-10.6	Saccharose Arice nut		
34	1.051	1.028	1.031		1.024	8.5-6.3	Concentrated plasma	1.020	90 we d
35	1.047	1.014	1.018		1.016	7.7-8.4	Saccharose	1.018	40

* After eschatin

† Red count.

‡ Maximum of 9 tests after March 1 1938

reduced kidney mass, pituitrin does not lead to any increase in urinary specific gravity above that obtained by water deprivation. Yet in these urines, the concentration of salts was not high, so that this cannot be the limiting factor that Motzfeldt (59) has shown it to be in the normal animals. Apparently, lack of this hormone is not an important cause of this type of hyposthenuria. Nor did adrenal cortical hormone, which has been shown to affect sodium reabsorption, have a detectable effect on specific gravity in these dogs.

Intravenous injection of large doses of sodium sulphate after 24 hours without water led to a urine exceeding the maximum postoperative concentration test by 0.01 or more in four of six experiments. In one (Dog 3) the gravity was as high as the maximum preoperative test, and in two others (Dogs 4 and 8) was 0.006 and 0.004 below the mean preoperative value. These urines were extremely high in sulphates, 75 to 85 per cent of the elevation of the specific gravity above that of water being accounted for by the sulphate present.

More significant physiologically are the results of an increase in plasma colloids and of a low blood pressure. In four of seven experiments the urinary specific gravity obtained with increased plasma colloid was as high or higher than the mean concentration test before operation. In

another, the gravity was 0.019 higher than the maximum after operation and only 0.004 lower than the mean preoperative value. In the remaining two, in which there was no significant increase in gravity, an attempt had been made to increase plasma colloids by intravenous injections of acacia solutions, and there was a reasonable doubt whether the doses given were large enough. Similarly, the specific gravity of the urine excreted at very low blood pressure, while small in volume, exceeded the maximum postoperative concentration test by 0.011 to 0.021 (average 0.016) and in two animals was as high as the mean preoperative concentration test gravity, while in the others it fell 1 to 4 points below this level.

The reduction in clearance is not directly proportional to the reduction in kidney mass, nor to the percentage of glomeruli remaining. Figure 3 shows the relation between the per cent of the original glomerular equipment of the animal remaining after operation and the per cent reduction in creatinine and urea clearances below the control level. The clearances are reduced less rapidly than the number of glomeruli, the difference being most marked with the smaller kidney fragments. This might be due to opening up and the more continuous activity of an increasing percentage of the total number of remaining glomeruli, until with extreme reduction in the

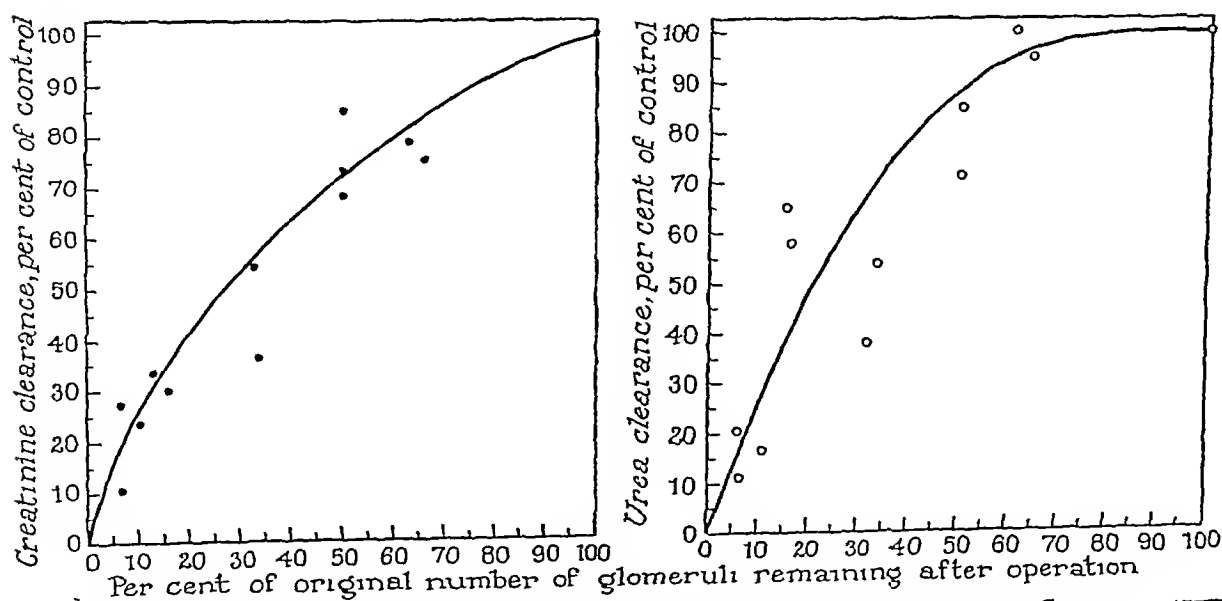


FIG 3 RELATION BETWEEN REDUCTION IN THE NUMBER OF GLOMERULI AND THE REDUCTION IN CLEARANCES AFTER SUBTOTAL NEPHRECTOMY

total number all which remained were continuously open, or to an increased amount of filtrate per open glomerulus. It is probable that both mechanisms are active. The former offers a reasonable explanation for the slight reduction in clearances when the number of glomeruli are reduced to 50 per cent of the original with no increase in blood nitrogen. When however, the kidney mass has been reduced to a point (about 35 per cent of glomeruli in these animals) where blood urea nitrogen is increased it seems not unreasonable to assume that the diuretic, vasodilator effect of the urea would lead to the constant perfusion of all the remaining glomeruli. Yet with still further reduction in the number of glomeruli the difference between the degree of glomerular reduction and decrease in clearances is greater. When the glomeruli were reduced to about 35 per cent, the average creatinine clearance was 46 per cent of control level while when the glomeruli were reduced to 5 to 15 per cent (average 9.4 per cent) the creatinine clearance was only reduced to an average of 23.8 per cent. This is similar to Verney's structural and functional reserve. The smaller reduction in urea than in creatinine clearance is consistent with this interpretation since with diuresis—increased volume flow down each tubule—there would be less back diffusion. The difference in creatinine and urea clearances is more difficult to explain on the basis of a simple perfusion of more glomeruli. Rhoads, Alving, Hiller and Van Slyke (69), and Levy and Blalock (48), found a relative increase in renal blood flow after unilateral nephrectomy, but this might be due either to perfusion of more glomeruli or to a greater blood flow per glomerulus. Medes and Herrick (56) found that the creatinine clearance paralleled the blood flow as measured by the Stromuhr. If applied to these animals, it would indicate a 25 to 85 per cent greater flow per glomerulus in the kidney remnant than in the kidney of the normal animal if it is assumed that in the latter all the glomeruli were open. The data do not support the hypothesis that the increase in systemic pressure is an important factor in producing the polyuria, for there is no relation between the degree of blood pressure elevation and the percentage increase in urine volume on concentration test. On the other hand, changes in glomerular capillary pressure resulting

from local vascular adjustments within the kidney perhaps influenced by increased concentrations of various substances in the plasma may well be of extreme importance.

Histologically the kidney fragments failed to show the tubular dilatation and flattening of epithelium described by Mark (52) or the degenerative lesions found by Anderson (6) in rabbits. Except in Dog 19 comparison of sections from the kidney remnant and the normal kidney did not show any striking difference. In the dogs allowed to survive several months there was apparently some increase in the size of the glomeruli. None showed any glomerulitis with hematoxylin and eosin stain. Since all of the kidneys had been injected the Heidenhain Mallory stains were unsatisfactory. In some kidneys there was slight tubular degeneration but this was evident in the intact kidney as often as in the kidney remnant. Sections of the kidney remnant from Dog 19 showed definite glomerular fibrosis and glomerulitis. There was also some widening of the tubules but no definite flattening of epithelium.

Summary Removal of sufficient renal mass thus leads to the excretion of an increased volume of dilute urine. The animals show a low specific gravity on concentration test, reduced urea and creatinine clearances, with variable elevation in blood pressure and blood urea nitrogen. The picture, as Volhard has emphasized, closely resembles that of nephrosclerosis, and differs from that of chronic glomerular nephritis chiefly in the absence of anemia and hematuria. Under certain conditions especially increase in concentration of plasma proteins and reduction in blood pressure, which there is no reason to believe lead to improvement in the condition of the tubule cells, urine can be obtained which equals or approaches in specific gravity that of the intact animal. The deduction seems logical that abnormality of renal epithelium is not the primary cause of the hyposthenuria.

Uranium poisoning

The histological and functional changes produced by uranium have been reviewed by MacNider (51).

There is an initial polyuria. With large doses this is followed by a decrease in urine formation and finally anuria with small doses recovery gradually takes place. The essential and dominant lesion in the kidney is injury to the epithelium of the tubules especially to the distal portion of the proximal convolutions, according to Suzuki (79). In animals which recover the tubular epithelium

is replaced by a flattened abnormal type. The urine is dilute and frequently contains sugar. Some workers report increased excretion of chloride and nitrogen, others a decrease. Phenolsulphonphthalein excretion is diminished. There is a retention of nitrogen, creatinine, etc., in the blood. Various changes in the glomeruli and in the reaction of their arterioles have been described, but the perfusion experiments of Ghoreyeb (29) and the blood flow measurements of Tribe, Hopkins, and Bar-

croft (83) indicate that there is no decrease in renal blood flow.

The dose of uranium acetate used in these experiments varied from 1 to 3 mgm per kgm. With the larger doses, some of the animals died before all the desired observations could be made. Table V and Figure 2 show the increase in urine

TABLE V
Summary of data on animals subjected to uranium poisoning and ureteral obstruction†

Dog	Weight	Before injury							After injury							Kidney weight and glomerular count	
		Concentration test		Mean clearance			Blood urea nitrogen	Mean blood pressure	Concentration test		Mean clearance			Blood urea nitrogen	Mean blood pressure	Left	Right
		Volume Mean Range	Specific gravity Mean Range	Inulin	Creatinine	Urea			Volume Mean Range	Specific gravity Mean Range	Inulin	Creatinine	Urea				
	kgm			cc per minute	cc per minute	cc per minute	mgm per cent	mm Hg			cc per minute	cc per minute	cc per minute	mgm per cent	mm Hg	grams thousandths	grams thousandths
URANUM POISONING																	
7	10.3	58 45-70	1.048(2) 1.040-55		43.8	33.2	7.4		663 500-980	1.017(3) 1.013-19		2.1	1.4	140		30.5 406	
13	10.8	97 93-104	1.044(3) 1.044-44		45.2	25.9	11.9	124	410 101-800	1.017(3) 1.013-19		21.7	3.4	57.5	110	66.5 344	55.0
14	13.2	123 86-160	1.038(2) 1.031-45		35.4	22.4	15.0	122	558 500-615	1.015(3) 1.013-17		12.3	3.6	70.5	122	34.8 322	35.0
28	9.6	87 63-105	1.042(4) 1.036-47	38.9	37.7	20.8	13.6	130	210 160-260	1.016(2) 1.013-18	3.05	2.3	2.1	68.2	140	29.3 259	33.0 266
39	7.0	51 17-91	1.048(5) 1.042-53	38.2	37.6	24.1	15.2	125	440	1.012	20.6	14.6	11.0	27.0	110	19.0	19.1
45	8.0	106 92-120	1.040(2) 1.040-41	44.4	43.2	18.2	23.0	135	388 360-415	1.009	2.7	2.2	1.7	61.0	140	18.0 369	19.0
46	8.2	64 62-65	1.055(2) 1.054-56	45.5	45.3	24.4	21.4	143	148 120-175	1.019(2) 1.013-22	3.0	2.3	1.9	96.6	150	23.5	21.8 278
64	12.7	149 142-156	1.036(2) 1.030-41	48.9	51.2			130	525 450-600	1.013(2) 1.011-15	5.2	4.2			130		
URETERAL CLAMP																	
22	12.4	75 69-81	1.033(2) 1.032-33		57.5	32.1	9.2	142	150 one lost	1.019(2) 1.015-23		3.0	1.7	130.0	145	48	46
26	7.5	90 60-120	1.052(2) 1.050-54		41.9	18.8	13.3		192 104-285	1.023(2) 1.022-23		9.7	7.5	31.6	150	45	63
34	7.0	50 32-78	1.051(5) 1.041-67		36.2	24.1	11.2	133	134 106-168	1.023(4) 1.022-26		22.7	16.7	20.6	135	40 238	42.5 152
35	9.1	42 35-48	1.047(2) 1.046-48		30.9	17.4	12.3	157	100 48-126	1.015(3) 1.014-17		18.3	8.3	34.0	135	43 350	44 365
38	7.7	65 52-86	1.041(4) 1.035-47		21.2	10.5	19.5		150	1.015		2.7	1.9	93.8			
28	10.0	57 50-62	1.052(3) 1.047-56		31.8	14.2	13.6	135	38 35-44	1.026(2) 1.024-28 1.032(3) 1.031-33 1.042(3) 1.036-47		0.87 24.4*	0.78 13.7	39.9 14.2	162 130	29.3 259	33.0 266
39	5.4	46 22-67	1.044(3) 1.041-50		43.7	28.5	14.4		218 210-225 91	1.015(2) 1.010-19 1.042		16.1 44.5*	15.6 19.1	28.4 25.1	125	19.1	19.0

† Figures in parentheses indicate the number of observations averaged.
* After removal of clamp.

volume and decrease in specific gravity on concentration tests, and the reduction in clearances after poisoning. There was no rise in blood pressure in any of these animals. Table IV shows the general failure of the methods yielding a more concentrated urine after subtotal nephrectomy to do so after uranium poisoning. No means have been found which will enable even the moderately poisoned kidney to put out a urine of high gravity. Some of the animals shown in Table IV have been omitted from Table V to save space, since clearances were not done after poisoning.

The histological evidence of damage to tubular epithelium permits the assumption that the polyuria and low specific gravity are due to impairment of water reabsorption, though whether this is chiefly in proximal or distal tubule is not indicated. The mechanism of the low clearances, however, requires further scrutiny. Diminished clearance might be due to back diffusion of the test substance through the damaged tubular epithelium, to diminished renal blood flow or to decrease in the permeability of the glomerular epithelium. The lack of histological evidence of glomerular thrombi, the marked increase in urine volume, and the increase in albuminuria make the last explanation unlikely. The experiments of Ghoreyeb and Tribe mentioned above, and those of Dunn, Dible, Jones and McSwiny (23) in oxalate poisoning indicate that there is no decrease in renal blood flow. However to confirm this point blood flow was measured by Barcroft's method in two animals after poisoning. These showed flows within the normal range for this method, but did not permit measurements in the same animal before and after poisoning. In two other animals blood flow was measured by the method of Van Slyke, Rhoads, Hiller and Alving (88) using a modification of their technique (31) of explanting the kidney so as to be certain that the samples of renal vein blood were not contaminated by arterial blood or urine. Creatinine and inulin were used as test substances. Samples of blood were drawn from femoral artery and renal vein before the beginning of urine collections, and at the end of each period. From analyses of blood samples curves were drawn for arterial and renal vein concentrations during the time of the experiment, and the values at the middle of each collection period estimated. Plasma flow was calculated as clearance ÷ per cent extraction blood flow from the hematocrit value. Table VI shows the results obtained. In control experiments inulin and creatinine clearances agree reasonably well. There is more discrepancy in the values for blood flow as calculated from inulin and creatinine. Small errors in extraction ratios make large differences in calculated blood flows. The agreement seems sufficient, however, to furnish acceptable evidence that there was no decrease in glomerular blood flow after poisoning. The marked decrease in A-V difference in Dog 64 after poisoning is striking. This must be due either to diminished glomerular permeability or

to much of the filtered substance having re-entered the blood stream through the tubule cells. Reasons have been given for believing that the former is not the mechanism; another reason for this belief is that the average extraction ratio for inulin is greater than for creatinine so that the glomerular membrane would have had to become more permeable to the large inulin molecule than to the smaller creatinine. After poisoning there is also a marked drop in the creatinine/inulin clearance ratio. Dog 65 was first given 0.5 mgm. per kgm. of uranium acetate to see if with the smaller dose there would be a decrease in creatinine clearance without much change in inulin clearance. This did occur but the difference was slight. Two weeks later she received an additional 1.0 mgm. per kgm. and another experiment was carried out after two days. This showed some further depression in creatinine clearance and in creatinine/inulin clearance ratio but again the creatinine clearance is not markedly altered. It seems that if the tubules are sufficiently damaged to permit any considerable back diffusion of creatinine, the large inulin molecule will also regain the blood stream by the same mechanism but to a lesser extent than creatinine (Dogs 28 39 45 46 Table V). These experiments indicate that with tubular damage a diminished extraction ratio may account for reduced clearances a mechanism suggested by Van Slyke *et al.* (87) but for which they had no direct evidence at the time.

The kidneys from these animals showed histological changes similar to those repeatedly described after uranium poisoning. There was no obstruction to perfusion flow, the glomeruli were well injected, and showed no consistent abnormality. The tubules, especially the proximal convoluted segments were the site of degeneration and necrosis. The severity of the tubular lesion varied with the dose, length of survival and in different animals receiving the same treatment.

Summary A predominantly tubular lesion with no reduction in blood flow can result in reduced clearances, and the excretion of an increased volume of dilute urine. In contrast to animals in which a similar decrease in renal function had been brought about by a reduction in renal mass, no conditions could be found under which these animals excreted a more concentrated urine. The most obvious explanation is that the damaged tubular epithelium was not only unable to reabsorb glucose, and to establish the normal osmotic gradient between lumen and capillary by reabsorption of water, but also permitted an abnormally great back diffusion not only of urea and creatinine, but even of the large inulin molecule. How far this process is simple diffusion, and how far it is influenced by the osmotic pressure of the plasma proteins in the peritubular capillaries, can not be analyzed further from the data at hand.

Ureteral obstruction

This method was used to simulate the conditions encountered in prostatic hypertrophy, stone, and hydronephrosis. Urethral obstruction would have been better, but would have precluded catheterization.

Suter (78) believed two factors contribute to the polyuria in such cases, tubular damage and "nervous reflex." Hinman and Hepler (36) believed that while excretory back pressure is the essential factor in producing hydronephrosis, its effect is closely linked with nutritional disturbances, and that the tubular atrophy is due more to anemia than to pressure. When the ureter is obstructed, constriction of renal artery gives a more rapidly developing hydronephrosis and atrophy than ureteral constriction alone (37). Through the kindness of Dr Goldblatt, his clamps and instruments were available. Under ether anesthesia a small midline incision was made just above the symphysis, and a clamp applied to each ureter close to the bladder. These were adjusted so as to constrict the ureter markedly, but not to occlude it. Since a slowly developing or marked hydronephrosis is associated with reduction in the number of glomeruli (46, 57) the effort was made to produce a lesion and carry out the observations as rapidly as possible.

After 5 to 11 days, these animals showed a definite impairment of concentrating ability, and reduction in clearances. There was no elevation in blood pressure. The degree of impairment was similar to that after uranium poisoning. Urine volumes, however, were not so uniformly increased (Figure 2). At times the volume of urine during a concentration test would be as low as during the control period, although the specific gravity was always lower. Table IV shows the results of attempts to obtain a concentrated urine by the means previously employed. After pituitrin a significant increase in specific gravity was obtained in one animal (Dog 30), increases of 2 to 5 points in four others. In no instance, however, was urine obtained of a concentration approaching the mean specific gravity on concentration test before obstructing the ureters. Administration of sulphate, increasing plasma protein concentration, and lowering the blood pressure were likewise without significant effect.

The kidneys showed some dilatation of the pelvis, but save in the left kidney of Dog 21 no marked atrophy of the renal cortex. Histologically, the glomeruli appeared normal. The tubular cells showed more or less evident cloudy swelling. In about half the kidneys, there was a secondary pyelonephritis.

That the tubular damage is a reversible process, from which recovery can take place, is shown in Dogs 28 and 39, in which the clamps were removed after reduction in clearances and low specific gravities had been secured. Functional recovery was complete or nearly so in a month.

TABLE VI
Renal blood flow before and after uranium poisoning

Dog	Inulin			Creatinine			Cell volume	Blood flow			Remarks
	Clearance	A-V difference		Clearance	A-V difference			From inulin	From creatinine	Creatinine Inulin	
		cc. per min. -ute	mgm.		per cent	cc. per min. -ute					
64	49.5			53.0						107	Control
	45.7	30	31.5	45.4	2.3	24.7	20.0	181	230	0.99	
	51.6	21	29.0	57.2	2.6	29.1	20.0	223	246	1.11	
	5.2	15	4.0	4.4	0.7	2.8	23.8	170	201	0.84	After uranium
	5.3	10	2.8	4.2	0.6	2.5	24.0	246	224	0.80	
	5.0	8	2.4	4.2	0.4	1.7	25.5	276	324	0.84	
65	37.4	50.0	31.2	36.0	4.0	26.3	38.2	245	221	0.97	Control
	39.7	33.5	29.5	38.8	3.2	23.6	37.1	211	256	0.98	
	36.2	63.0	26.2	33.2	4.7	25.3	36.1	216	206	0.92	Twenty two days after right nephrectomy
	32.5	44.0	26.5	30.2	4.4	25.3	38.4	198	192	0.93	
	35.3	30.0	25.0	33.1	4.0	24.3	36.1	220	212	0.94	
	35.0	50.0	23.5	31.9	3.8	20.8	35.3	230	237	0.91	After 0.5 mgm. per kgm. of uranium acetate
	36.3	38.0	25.0	29.1	4.2	23.8	35.8	226	190	0.86	
	38.2	28.5	24.2	32.9	4.1	24.9	36.4	249	208	0.86	
	32.9	48.0	27.0	25.5	4.8	24.4	29.7	174	149	0.78	After 1 mgm. per kgm. of uranium acetate
	35.9	25.5	23.4	26.4	4.3	23.5	33.0	228	168	0.74	
	27.0	20.0	24.7	21.3	3.7	22.1	35.5	169	149	0.79	

Summary The explanation for the loss of concentrating power offered for uranium poisoning would seem to be applicable here also. Reduction in blood flow, however, may be a factor in the reduced clearances. Levy, Mason, Harrison, and Blalock (49) found reduced blood flow when the ureters were tied. The gradual reduction in the concentration of urea, increase in chloride, and appearance of sugar in the fluid obtained from a hydronephrotic sac, indicates the tubules have not only lost the capacity to concentrate urea, but to reabsorb chlorides and sugar to the normal extent.

Renal denervation

Claude Bernard (11) in 1859 found that division of the splanchnic nerves on one side led to an increased volume of urine from the ipsilateral kidney. This has been confirmed repeatedly.

Marshall and Kolls (54) cite the literature up to the time of their papers. An increase in renal blood flow is usually offered as the mechanism responsible for the

diuresis although Bayliss and Fee (10) found that in the double heart lung-kidney preparation, while splanchnic section increased blood flow pituitrin decreased the urine volume without any change in blood flow. Rhoads *et al* (70) did not find any increase in blood flow after splanchnic section. Denervated and normal kidney responded alike to ingestion of water exercise, pituitary extract (44) and to afferent nerve stimulation (81). Apparently the response of an animal with denervated kidneys to a concentration test has not been studied.

Complete denervation of the kidney can only be secured, according to Quinby (65) by section and resuture of artery vein, and ureter. Even under these conditions the diuresis disappears in about two weeks. Most authors have been content to divide all visible nerves entering the hilus or to cut the splanchnic nerves.

After preliminary concentration tests and measurement of water intake and urine excreted when water was allowed *ad lib* the kidneys of two dogs (Numbers 47 and 59) were denervated. Under ether anesthesia, the kidney was exposed through a lumbar incision, delivered into the wound, the capsule stripped of all adherent fat, and artery vein and ureter carefully cleared and finally wiped rather vigorously with gauze. All other structures entering the hilus were divided.

TABLE VII

Urine volumes and concentration tests before and after renal denervation*

Dog	Before denervation				After denervation				Specific gravity after pituitrin
	Mean urine volume	Concentration test		Mean urine volume	Concentration test				
		Mean urine volume	Mean specific gravity		Mean urine volume	Mean specific gravity			
47	102 (6)	74 (6)	1 035	278 (4)	93 (3)	1 020	1 035		
59	198 (6)	86 (2)	1 048	583 (4)	260 (2)	1 019	1 028		

* Figures in parentheses indicate the number of observations averaged

After operation, the daily urine volume when water was allowed was increased, and the specific gravity on concentration test reduced (Table VII). In one dog pituitrin yielded a urine of specific gravity equal to that of the mean concentration test gravity before operation in the other the increase after pituitrin was less marked. The mechanism of the polyuria and of the mode of action of pituitrin has not been studied. The experiments served only to show an experimentally

produced loss of concentrating power without other impairment of kidney function.

Anemia

Christian (20) and Mosenthal (58) have noted low gravity in patients suffering from pernicious anemia, with improvement during remission. Fouts and Helmer (26) reported low urea clearances with improvement on liver therapy. If this were due simply to anoxemia of the tubule cells it seemed that it should be reproduced in animals if hemoglobin was maintained at a low level by repeated bleedings. Since, in the dog hemoglobin regeneration is very rapid on the stock diet the animals were given either a bread and milk or Whipple's bread and salmon diets. On these diets alone, concentration test specific gravities are lower than on the stock diet. After bleeding the plasma was separated and re injected in order to maintain plasma proteins at a normal level. If necessary additional plasma was supplied from normal dogs. Dog 33 showed no impairment of concentrating power or decrease in creatinine clearance after her hemoglobin had been reduced from 16 grams per 100 cc. to approximately 3.9 grams and maintained at that level for a month. Dog 42 was maintained at a level of about 7.8 grams per 100 cc. for two and a half months, and then at from 4.6 to 5.9 grams per 100 cc. for an additional month. While during this time some concentration tests showed gravities as low as 1.020 others were well within the range of those obtained during the control period. The same was true of Dog 49 whose hemoglobin was maintained at 2.5 to 3.9 grams for a month. These dogs did not show any significant change in creatinine or urea clearances or in blood pressure during the period of anemia.

While the number of experiments is small they indicate that the mechanism of the diminished renal function in pernicious anemia may not be due to the low hemoglobin alone, or if it is, the anemia must be present for a longer time than it has been maintained in these animals.

Through the kindness of Dr Goldblatt, an opportunity was offered to study the concentrating ability of some of his dogs with experimental hypertension produced by renal ischemia. In Table VIII are shown the maximum specific gravities on concentration tests and the mean blood pressure before and after constriction of

the renal arteries or of the aorta above the renals. It is evident that hypertension can be produced by these means without any reduction in the ability of the kidney to excrete a concentrated urine. The renal blood flow and intrarenal blood pressure are probably reduced in these animals. As long as the reduction in flow is not sufficient to interfere with the nutrition of the tubule cells, a concentrated urine is to be expected. Animals with the "malignant" type of hypertension have not been studied.

TABLE VIII

Mean blood pressure and maximum specific gravity of urine on concentration test in Dr Goldblatt's dogs before and after the production of renal ischemia

Dog	Control period		After production of renal ischemia	
	Mean blood pressure	Maximum specific gravity	Mean blood pressure	Maximum specific gravity
235	140	1.042	170	1.038
240	135	1.047	190	1.045
330	120	1.050	192	1.053
340	125-140	1.037	177	1.046
344	120	1.035	195	1.040
368	130	1.027	210	1.040

Dr T Birch generously allowed observations on two of his animals during development of black tongue on a vitamin B₆ deficient diet and its cure by nicotinic acid. No change in the ability to excrete a urine of high specific gravity on a concentration test during these nutritional changes was noted.

Two dogs happened to be pregnant at the time observations were started. One failed to attain a normally high specific gravity during the last month of pregnancy, and both failed for a month after whelping. Subsequently, both excreted urines of normal concentration.

The lower test specific gravities on a low protein diet are not necessarily due to the same mechanism as the reduction in clearances. Pitts (64) and Herrin, Rabin, and Feinstein (35) have suggested that the latter is related to the level of protein metabolism, and have shown that ingestion of salts or urea are without effect. Dog 50 had a mean specific gravity of 1.026 for three tests after a month on the cracker meal diet. She was then given 10 grams of urea and 5 grams of NaCl and put in a metabolism cage. The urine volume for the following twelve hours was 210 cc. and its specific gravity 1.028, for the next twelve hours the volume was 130 cc. and the specific gravity 1.046. After 40 units of pituitrin she excreted a urine having a specific gravity of 1.051. Dog 52, which showed a mean specific gravity of 1.037 for six tests on a bread and salmon diet, excreted a urine of 1.050 after 12 grams of urea and 5 grams of NaCl. Her blood urea rose from 12.0 to 19.5 mgm. per cent. After pituitrin, however, the highest gravity obtained during the succeeding twelve hours was 1.041. Dog 53, which had shown a decrease from 1.061 on stock diet to 1.026 on

cracker meal during the winter, still had a mean of 1.047 for six tests with one as high as 1.060 after a month of cracker meal diet during the summer.

DISCUSSION

These experiments confirm many in the literature in demonstrating that there are several ways in which loss of concentrating power may be brought about. These include reduction in the number of nephrons, tubular poisoning, tubular degeneration resulting from back pressure, or back pressure plus ischemia, (temporarily at least) interference with the nerve supply to the kidney, at times low protein diet, and possibly pregnancy. Lesions in the mid-brain, producing diabetes insipidus should be included. Three of these are associated with other evidence of renal impairment, diminished clearances and elevation of blood urea and creatinine. Only one exhibits any tendency to be associated with hypertension (reduction in kidney mass). Some are reversible processes (diet, pregnancy, denervation) from which restoration to normal regularly occurs. Others (tubular damage) may recover if the injury has not been too severe. Reduction in kidney mass is irreversible, and while hypertrophy of the remaining tissue may be accompanied by some improvement in function, this is not apparent when renal mass has been reduced beyond a certain point.

The response of animals with these different types of hyposthenuria to various attempts to obtain a concentrated urine differs. Pituitary extract leads to a urine of normal specific gravity after renal denervation, low protein diet, and (from the literature) after lesions of the mid-brain, it is without significant effect in tubular damage and with reduction in kidney mass. In the latter, increase in the concentration of plasma colloids and reduction of blood pressure to near the critical level lead to the excretion of a more concentrated urine. In the presence of tubular damage these are without effect.

The urine volume in all tends to be above normal except in advanced tubular degeneration, when it is diminished, or even suppressed. In uranium poisoning, the presence of a normal blood flow with a diminished extraction ratio, which is lower for creatinine than for inulin, indicates that not only has the ability of the tubules to reabsorb

sugar and water been impaired, but also the ability to prevent such substances as creatinine concentrated to some extent by reabsorption of water, from re entering the blood stream. The oliguria or anuria in extreme damage seems explicable on the assumption that these severely damaged cells act like a dead membrane, and that the glomerular filtrate may be completely reabsorbed, the absorbing force being the osmotic pressure of the plasma colloids in the peritubular capillaries. This occurrence was demonstrated by Richards in frogs anuric from mercuric chloride poisoning (71).

Hyposthenuria results from lesser degrees of tubular damage because of the loss of capacity of the damaged cells to reabsorb water to the normal degree against the increasing osmotic pressure of the fluid in the lumen of the tubules. Accompanying this there is loss of the ability to resist back diffusion of substances concentrated by the reabsorption of water. This back diffusion is greater for urea than for creatinine, and greater for creatinine than for inulin. Evidence is not available to decide the relative importance of the two factors, nor the parts of the tubule involved. When the tubules are severely damaged, most or all of the glomerular filtrate is reabsorbed, the little urine that is excreted approaching an ultrafiltrate of plasma in composition. With lesser degrees of tubular damage there is an increased volume of dilute urine. As the degree of damage increases the urine volume diminishes but remains dilute. No means have been found which will permit the excretion of a concentrated urine from such kidneys.

The polyuria and hyposthenuria resulting from decreased kidney mass is adequately explained by increased blood flow and greater volume of filtrate per remaining glomerulus. This results in a more rapid flow of fluid down the tubule, lack of time for reabsorption accounting for the hyposthenuria. Under suitable conditions a small kidney remnant excretes a urine of high specific gravity. There is no evidence in the experiments presented here that continued polyuria exhausts or damages the tubular cells. It seems unnecessary therefore to assume tubular damage in addition to the circulatory changes in order to explain this polyuria.

Other mechanisms for hyposthenuria, such as disturbances in circulating hormones undoubtedly

exist but sufficient evidence is not at hand to make discussion profitable.

Hyposthenuria might be classified as renal and extrarenal or parenchymal and extraparenchymal, or tubular and non tubular. In the former category belong the definite tubular degenerations in the latter, reduction in renal mass. Sufficient evidence is not yet available to know where to place the hyposthenuria of denervation, low protein diet, and of pregnancy.

CONCLUSIONS

Hyposthenuria, or loss of ability to excrete a concentrated urine under usual conditions, may be produced experimentally in dogs in a number of ways. These include reduction in kidney mass, uranium poisoning, ureteral obstruction, denervation and a low protein diet.

Dogs subjected to subtotal nephrectomy will excrete a concentrated urine under certain conditions, including increased concentration of plasma colloids, low blood pressure, and injections of sodium sulphate after water deprivation. A urine of high specific gravity has not been obtained from dogs with tubular damage. Pituitrin leads to the excretion of a concentrated urine after renal denervation but is without significant effect in the other groups.

BIBLIOGRAPHY

1. Adams, L. J., Egloff, W. C., and O'Hare, J. P., Experimental chronic nephritis produced by radium. *Arch. Path.*, 1933 15 465.
2. Addis, T., and Foster, M. G., The specific gravity of the urine. *Arch. Int. Med.*, 1922, 30 555.
3. Akerrén, Y., Die Funktionsweise der Schrumpfnieren im Lichte der Cushnyschen Harnbildungs-theorie. *Acta med. Scandinav.*, 1927 66, 524.
4. Allen, F. M., Scharf, R., and Lundin, H., Clinical and experimental renal deficiency. *J. A. M. A.*, 1925 85 1698.
5. Alving, A. S., and Van Slyke, D. D., The significance of concentration and dilution tests in Bright's disease. *J. Clin. Invest.*, 1934 13 969.
6. Anderson, H., Experimental renal insufficiency. *Arch. Int. Med.*, 1926 37 297.
7. Applebach, C. W., and Jensen, C. R., Experimental chronic renal insufficiency in dogs with special reference to arterial hypertension. *J. Clin. Invest. (Proc.)* 1931 10 162.
8. Bainbridge, F. A., and Beddard, A. P., The relation of the kidneys to metabolism. Preliminary communication. *Proc. Roy. Soc. London, S.B.*, 1907 79 75.

- 9 Bartels, C., Diseases of the Kidney "Ziemssen's Cyclopaedia of the Practice of Medicine." Vol 15 Wm Wood, New York, 1877
- 10 Bayliss, L. E., and Fee, A. R., Studies on water diuresis III A comparison of the excretion of urine by innervated and denervated kidneys perfused with the heart-lung preparation J Physiol, 1930, 69, 135
- 11 Bernard, C., Leçons sur les Propriétés Physiologiques et les alterations pathologiques des Liquides de l'Organisme. Bailliere, Paris, 1859
- 12 Blackall, J., Observations on the nature and cure of dropsies, and particularly on the presence of the coagulable part of the blood in dropsical urine James Webster, Philadelphia, 1820 (1st Am. edition from 3d English edition.)
- 13 Bradford, J. R., The results following partial nephrectomy and the influence of the kidney upon metabolism. J Physiol, 1898, 23, 415
- 14 Bright, R., Reports of medical cases selected with a view of illustrating the symptoms and cure of diseases by a reference to morbid anatomy 1827 London. (Published in Original Papers of Richard Bright on Renal Disease. Oxford University Press, London, 1937)
- 15 Cash, J. R., A preliminary study of the blood pressure following reduction of renal substance, with a note on simultaneous changes in blood chemistry and blood volume. Bull Johns Hopkins Hosp, 1924, 35, 168
- 16 Chanutin, A., Experimental renal insufficiency produced by partial nephrectomy III Diets containing whole dried liver, liver residue and liver extract. Arch Int Med, 1934, 54, 720
- 17 Chanutin, A., and Ferris, E. B., Jr., Experimental renal insufficiency produced by partial nephrectomy I Control diet. Arch Int. Med., 1932, 49, 767
- 18 Chanutin, A., and Ludwig, S., Renal function studies in partially nephrectomized rats J Biol Chem. (Proc.), 1935, 109, xviii
- 19 Chasis, H., and Smith, H. W., The excretion of urea in normal man and in subjects with glomerulonephritis J Clin. Invest., 1938, 17, 347
- 20 Christian, H. A., Renal function in pernicious anemia as determined by dietary renal tests Arch Int. Med., 1916, 18, 429
- 21 Christison, R., On granular degeneration of the kidneys and its connection with dropsy, inflammation, and other diseases (Dunglison's Am. Med. Library.) A. Walde, Philadelphia, 1839
- 22 de Paoli, E., Della resezione del rene. Zentralbl f Chir, 1892, 19, 78
- 23 Dunn, J. S., Dible, J. H., Jones, N. A., and McSwiney, B. A., The renal circulation rate in experimental oxalate nephritis J Path and Bact, 1925, 28, 233
- 24 Ferris, H. W., and Hynes, J. F., Indirect blood pressure readings in dogs, description of method and report of results J Lab and Clin. Med, 1931, 16, 597
- 25 Fishberg, A. M., Hypertension and Nephritis Lea and Febiger, Philadelphia, 1934, 3d ed
- 26 Fouts, P. J., and Helmer, O. M., Urea clearance in pernicious anemia Arch Int. Med, 1938, 61, 87
- 27 Fremont-Smith, F., Fremont-Smith, M., Dailey, M. E., Solomon, P., Stetten, DeWitt, Jr., and Carroll, M. P., Studies in edema I The mechanism of water diuresis in man J Clin Invest. (Proc.), 1930, 9, 7
- 28 Gamble, J. L., McKhann, C. F., Butler, A. M., and Tuthill, E., An economy of water in renal function referable to urea Am J Physiol, 1934, 109, 139
- 29 Ghoreyeb, A. A., A study of the mechanical obstruction to the circulation of the kidney produced by experimental acute toxic nephropathy J Exper Med, 1913, 18, 29
- 30 Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., Studies on experimental hypertension. I The production of persistent elevation of systolic blood pressure by means of renal ischemia J Exper Med, 1934, 59, 347
- 31 Gordon, W., Alving, A. S., Kretzschmar, N. R., and Alpert, L., Variations in the extraction of urea by the kidney and their relation to the amount of urea reabsorbed Am J Physiol, 1937, 119, 483
- 32 Govaerts, P., Le Fonctionnement du Rein Malade. Masson & Cie, Paris, 1936
- 33 Hartman, F. W., Methods and effects of increasing the urinary constituents in the body J Exper Med, 1933, 58, 649
- 34 Hayman, J. M., Jr., and Starr, I., Jr., Experiments on the glomerular distribution of blood in the mammalian kidney J Exper Med, 1925, 42, 641
- 35 Herrin, R. C., Rabin, A., and Feinstein, R. N., The influence of diet upon urea clearance in dogs Am J Physiol, 1937, 119, 87
- 36 Hinman, F., and Hepler, A. B., Experimental hydro-nephrosis, the effect of changes in blood pressure and blood flow on its rate of development I Splanchnotomy Increased intrarenal blood pressure and flow, diuresis Arch Surg, 1925, 11, 578
- 37 Hinman, F., The Principles and Practice of Urology W B Saunders Co, Philadelphia, 1935
- 38 Janeway, T. C., A modification of the Riva-Rocci method of determining blood pressure for use on the dog Proc. Soc. Exper Biol and Med, 1909, 6, 108
Nephritic hypertension, clinical and experimental studies Am. J. M. Sc., 1913, 145, 625
- 39 Jensen, C. R., and Apfelbach, C. W., Method of making repeated determinations of intra-arterial systolic blood pressure in dogs Arch Path, 1928, 6, 99
- 40 Joelson, J. J., Beck, C. S., and Moritz, A. R., Renal counterbalance. Arch Surg, 1929, 19, 673
- 41 Johnson, G., Lumlilan lectures on the muscular arterioles Brit. M. J., 1877, 1, 443
- 42 Jolliffe, N., and Smith, H. W., The excretion of

- urine in the dog II. The urea and creatinine clearance on cracker meal diet. *Am. J. Physiol.*, 1931 99 101
- 43 Karsner H. T., Bunker, H. A., Jr., and Grabfield, G. P., A note on the immediate effects of reduction of kidney substance. *J. Exper. Med.*, 1915 22, 544
 - 44 Klisiński, A., Pickford, M., Rothschild, P., and Verney E. B., The absorption and excretion of water by the mammal. II. Factors influencing the response of the kidney to water ingestion. *Proc. Roy. Soc., London s.B.*, 1933 112, 521
 - 45 Kunkel, P. A., Jr., The number and size of the glomeruli in the kidney of several mammals. *Bull. Johns Hopkins Hosp.*, 1930 47 285
 - 46 Landis E. M., Elsom, K. A., Bott, P. A., and Shiels, E., Observations on sodium chloride restriction and urea clearance in renal insufficiency. *J. Clin. Invest.*, 1935 14 525
 - 47 Lashmet, F. H., and Newburgh, L. H., An improved concentration test of renal function. *J. A. M. A.* 1932, 99 1396.
 - 48 Levy S. E., and Blalock, A., The effects of unilateral nephrectomy on the renal blood flow and oxygen consumption of unanesthetized dogs. *Am. J. Physiol.*, 1938, 122, 609
 - 49 Levy S. E., Mason M. F., Harrison, T. R. and Blalock A., The effects of ureteral occlusion on the blood flow and oxygen consumption of the kidneys of unanesthetized dogs. *Surgery* 1937 1, 238.
 - 50 Lundin, H., and Mark, R., Feeding of protein to partially nephrectomized animals. *J. Metabolic Research*, 1925 7 221
 - 51 MacNider W. B., A review of acute experimental nephritis. *Physiol. Rev.*, 1924 4 595
 - 52 Mark, R. E., Untersuchungen über die Nierenfunktion. Ergebnisse partieller Nierenarterienunterbindung am Hunde. *Ztschr. f. d. ges. exper. Med.*, 1928 59, 601
 - 53 Mark, R. E., and Gessendörfer H. Untersuchungen über die Nierenfunktion. Zur Frage des Zusammenhanges von Nierenmasse, Herzhypertrophie und Blutdrucksteigerung. *Ztschr. f. d. ges. exper. Med.*, 1930 74 350
 - 54 Marshall, E. K. Jr., and Kolls A. C. Studies on the nervous control of the kidney in relation to diuresis and urinary secretion. I. The effect of unilateral excision of the adrenal, section of the splanchnic nerve and section of the renal nerves on the secretion of the kidney. *Am. J. Physiol.*, 1919 49 302.
 - 55 Mayrs E. B. The functional pathology of nephritis. *Quart. J. Med.*, 1926, 19 273
 - 56 Medes G., and Herrick, J. F., Blood flow to the kidneys and creatinine clearance. *Proc. Soc. Exper. Biol. and Med.* 1933 31 116.
 - 57 Moritz, A. R., and Hayman, J. M. Jr., The disappearance of glomeruli in chronic kidney disease. *Am. J. Path.*, 1934 10, 505
 - 58 Mosenthal, H. O., Renal function as measured by the elimination of fluids salt and nitrogen, and the specific gravity of the urine. *Arch. Int. Med.*, 1915 16 733
 - 59 Motzfeldt, K., Experimental studies on the relation of the pituitary body to renal function. *J. Exper. Med.*, 1917 25 153
 - 60 Müller F., *Berechnung und Begriffsbestimmung auf dem Gebiet der Nierenkrankheiten.* Veröffentlich. a.d. Geb. d. Militärsondatsmens 1916 65 1
 - 61 Newman, D., The pathology of albuminuria in its relation to morbid structural changes in the kidney. *Glasgow M. J.* 1884 21, 190.
 - 62 Passler and Hemecke, Versuche zur Pathologie des Morbus brightii. *Verhandl. d. deutsch. path. Gesellsch.*, 1905 9 99
 - 63 Pilcher J. D., On the excretion of nitrogen subsequent to ligation of successive branches of the renal arteries. *J. Biol. Chem.*, 1913 14 389
 - 64 Pitts, R. F., The effect of protein and amino acid metabolism on the urea and xylose clearance. *J. Nutrition*, 1935 9 657
 - 65 Quinby W. C., The function of the kidney when deprived of its nerves. *J. Exper. Med.* 1916, 23, 535
 - 66 Rayer P. F. D., *Traité des Maladies des Reins et des Altérations de la Sécrétion Urinaire, étudiées en elles-mêmes et dans leurs Rapports avec les Maladies des Urèbres, de la Vessie, de la Prostate, de l'urèthre, etc. avec un Atlas in Folio* Vol. 2. Baillière, Paris 1840.
 - 67 Rehberg P. B. Ueber die Bestimmung der Menge des Glomerulussfiltrats mittels Kreatinin als Nierenfunktionsprüfung nebst einigen Bemerkungen über die Theorien der Harnbereitung. *Zentralbl. f. inn. Med.*, 1929 50 367
 68. Rehberg P. P., *The Kidney in Health and Disease.* Edited by H. Berglund and G. Medes. Lea and Febiger Philadelphia, 1935, p. 88.
 - 69 Rhoads, C. P., Alving A. S., Hiller A., and Van Slyke, D. D. The functional effect of transplanting one kidney and removing the other. *Am. J. Physiol.*, 1934 109 329
 70. Rhoads, C. P. Van Slyke, D. D., Hiller A., and Alving A. S., The effects of novocainization and total section of the nerves of the renal pedicle on renal blood flow and function. *Am. J. Physiol.*, 1934 110, 392.
 71. Richards, A. N., Direct observations of change in function of the renal tubule caused by certain poisons. *Tr. A. Am. Physicians* 1929 44 64
 72. Richards A. N., Westfall B. B., and Bott P. A., Renal excretion of inulin, creatinine and xylose in normal dogs. *Proc. Soc. Exper. Biol. and Med.*, 1934 32 73.
 73. Saundby R., *Lectures on Bright's Disease.* John Wright and Co., Bristol 1889
 - 74 Schlayer Hedinger and Takayasu, R., Über nephritisches Ödem. *Deutsches Arch. f. klin. Med.*, 1907 91 59

TABLE I

*Distribution of index cases of rheumatic and control families according to the correlation of the history of rheumatic manifestations in the parents and grandparents**

Grandparental history				Rheumatic index cases					Control index cases				
Paternal		Maternal		Parental history					Parental history				
GF	GM	GF	GM	F - M -	F + M -	F - M +	F + M +	Total	F - M -	F + M -	F - M +	F + M +	Total
-	-	-	-	23	3	9	2	37	25	3			28
-	-	+	-	9	1		2	12					
-	-	-	+	3	2	6		11	2				2
-	-	+	+	2		2		4					
+	-	-	-	5	1	1	2	9					
+	-	+	-										
+	-	-	+			1		1	1				1
+	-	+	+										
-	+	-	-	4	1	2	1	8	1				1
-	+	+	-	1				1					
-	+	-	+		1	1		2					
+	+	+	-										
+	+	+	+	1				1					
+	+	+	+										
+	+	+	+	1				1					
?	-	-	+	1		2		2					
?	-	+	+			1		1					
?	-	-	-	1				1					
?	?	-	-	1				1			1		1
?	?	-	+			2		2					
-	-	?	?	2				2					
Total				53	9	27	7	96	29	3	1		33

* F = father M = mother GF = grandfather GM = grandmother

It was possible to obtain complete histories with respect to the occurrence of rheumatic manifestations on every parent of all the rheumatic and control index cases, but the history with respect to rheumatic disease is complete in the grandparents of only 86 of the rheumatic and 32 of the control index cases. The discussion, therefore, of the proportion of index cases with history of rheumatic manifestations in their grandparents is limited to those cases for whom the information was complete.

Of the 96 rheumatic index cases, 43, or 44.8 per cent, had one or both parents with a history of rheumatic disease in the past as compared with 4, or 12.1 per cent, of the 33 control index cases. Thus the percentage of index cases with rheumatic parents in the rheumatic group was 37 times as high as that of the control group. The percentage of index cases who had grandparents with a positive history in the rheumatic group was almost five times that found in the control group, the percentages being 57.0 to 12.5.

When both parental and grandparental histories are considered, the percentage of index cases with at least one parent or grandparent giving a history of rheumatic manifestations was 73.3 per cent in the rheumatic group as compared with 21.9 per cent in the control group. These results show quite definitely that a much greater proportion of the rheumatic index cases have parents or grandparents who have had rheumatic disease than is found in the corresponding relatives of the control index cases. They demonstrate in another form the findings of the previous article, which showed that there was an unusual occurrence of this disease in the families of rheumatic index cases.

The findings with respect to the percentage of rheumatics who have parents with rheumatic histories is, moreover, consistent in the two generations analyzed. Forty-three of the 96 rheumatic index cases had parents with a history of rheumatic disease. A study of Table I shows that 36 of these had one parent rheumatic and 7 had

both parents rheumatic, or there were in all 50 rheumatic parents. A summary of the history of rheumatic manifestations in *their* parents (grandparents of the index cases) compiled from Table I shows for these rheumatic parents

Number of rheumatic parents of index cases	50
with rheumatic history in <i>their</i> parents	Number 23
	Percent 46.0

This percentage is in agreement with the percentage of index cases whose parents gave a history of rheumatic manifestations seen in the summary of Table I, and shows the consistency of this finding in two generations of these rheumatic families

The offspring of rheumatic and non rheumatic parents

The families in this study were selected in two ways, (1), those of the rheumatic index cases because at least one child the index case, came to the clinic with some form of rheumatic manifestation, and (2), those of the control group because the index case was examined in the Tuberculosis Clinic and had not had an acute rheumatic episode. Thus by definition, at least one child in each of the rheumatic families must be rheumatic, and one child in each of the control families must be non rheumatic.² Because of this method of selection the immediate families of the index cases are not suitable for a direct comparison of the relative frequency of rheumatic infection in the offspring of the parental matings

The information obtained for the grandparents and *their* children may, however, be used for this purpose, because selection of the families was entirely independent of any prior knowledge of the past history with respect to rheumatic manifestations of the grandparents, parents, uncles or aunts of the index cases. The findings of this analysis are, therefore, not comparable with the findings of other investigators who used the immediate families of their index cases to study this relation (2, 3, 4)

* It should be noted that control families were selected without reference to the past history of rheumatic manifestations in the siblings of the index case so that it could happen that some of the siblings had been registered in the Cardiac Clinic—this did occur in three instances

These grandparental families also have the advantage in that they are complete the grandparents are all past the reproductive age and their living offspring are mostly past the age of maximum incidence of rheumatic disease. It is also permissible to combine data on both the rheumatic and control families, because in this generation any difference which might be shown between the two groups is not due to the method of sampling but should rather be considered due to the selectivity of the disease.

The total number of grandparental families in the combined rheumatic and control groups was 258, and complete information was available with respect to the history of rheumatic manifestations in the parents and children of 246 of these families (see Table I). From the 246 matings, for which complete histories are available, there are 1303 offspring, of which 150 had a history of some type of rheumatic manifestation

The distribution of the grandparents of index cases according to their history of rheumatic disease, the number of offspring and the number and percentage of rheumatic offspring from each type of mating is shown by sex, in Table II

TABLE II
Distribution of grandparental families of index cases according to the history of rheumatic disease in the parents with the number of offspring of each type of mating and the number and percentage of rheumatic offspring, by sex

	History of rheumatic manifestations in parents of grandparental families (grandparents of index cases)					Total
	Male - Female	Male + Female	Male - Female	Male + Female	Unknown	
Number of matings.	183	34	34	6	12	259
Male offspring						
Number	491	61	55	18		655
Number rheumatic	21	6	10	6		61
Percentage rheumatic.	8.3	12.1	18.3	33.3		
Female offspring						
Number	453	40	104	21		618
Number rheumatic	42	7	24	6		79
Percentage rheumatic	8.7	17.5	22.7	28.6		

A study of this table shows that where one or both parents (grandparents of index cases) gave a rheumatic history a much higher percentage of the offspring (parents, uncles and aunts of index cases) were affected than when neither mate had manifestations. There are in addition, some interesting sex differences which are worthy of note. From the 34 matings of a rheumatic female with a non rheumatic male there were 104 female off

spring of whom 34 were affected, and 85 male offspring of whom 16 had manifestations. Rheumatic mothers thus had almost twice the percentage of rheumatic children among their female offspring than they had among their male offspring. This is in contrast to the fact that no difference is noted in the percentage of male and female offspring who were rheumatic in the 24 families where the father was rheumatic and the mother non-rheumatic. Viewed from another angle, we note with respect to the male offspring, that there was apparently no difference in the percentage who were rheumatic in the families in which the mother gave a positive history and those in which the father gave a positive history. Such was not the case with the female offspring, the percentage of female offspring who were rheumatic being almost twice as high in the families where the mother was rheumatic as it was in the families of rheumatic fathers.

The higher percentage of rheumatic children found among the offspring of rheumatic parents than among the offspring of non-rheumatic parents suggests that the hereditary constitution may be a factor in determining predisposition to this disease. The higher frequency of rheumatic disease among female offspring of rheumatic mothers would seem to indicate that if heredity is a factor a sex difference exists.

Rheumatic manifestations on the paternal and maternal sides of families of rheumatic index cases

A comparison of the percentage of the offspring of the paternal and maternal grandparents of the rheumatic index cases who gave a history of rheumatic manifestations yields further information upon possible hereditary relationships. In making such a comparison, the parents of the index cases must be excluded, because by the method of selection of these families, there must be one male child in each family of paternal grandparents and one female child in each family of maternal grandparents. Any hereditary factor present in these families would also manifest itself most strongly in the parents of the rheumatic index cases since if such a factor be present, they must, of necessity, be the transmitters of the disease.

Another consideration is the accuracy of the histories obtained, because it is generally much

easier to interview the mother than the father of the index case, and for this reason only those families on the paternal side were considered in which the history of rheumatic disease was obtained from the father or one of his sisters.

The histories of 86 paternal and 96 maternal families are believed to be accurate, and these have been analyzed to show the percentage of aunts and uncles (siblings of parents of index case) with a rheumatic history on the paternal as compared with the maternal side. This comparison is shown in Table III.

TABLE III

Comparison of the relative frequency of rheumatic manifestations in the aunts and uncles of rheumatic index cases on the paternal and maternal sides

Relation to index case	Paternal			Maternal		
	Number	Rheumatic		Number	Rheumatic	
		Number	Per cent		Number	Per cent
Uncles	159	4	2.5	213	33	15.5
Aunts	172	9	5.2	225	35	15.6

A study of this table reveals the interesting fact that while there is no difference between the proportion of the uncles and aunts who have rheumatic manifestations on their respective sides of the family, the percentage of maternal aunts and uncles who had rheumatic manifestations is almost three times that of the paternal aunts and uncles.

As these aunts and uncles enter the study because they are siblings of the parents of the index cases, any interpretation of the above finding is dependent to some extent upon whether the difference noted is due to the fact that in this group of families a greater number of mothers than fathers of index cases had a history of having had rheumatic disease (see Table I). A further analysis has therefore been made in which the aunts and uncles are classified as siblings of rheumatic and non-rheumatic parents to compare paternal and maternal sides of these families according to the history of the parent through whom they are related to the rheumatic index case. This comparison is shown in Table IV.

This table amplifies the findings shown in Table III and demonstrates the fact that, when due consideration is given to the presence or absence of a history of rheumatic disease in the parents of

TABLE IV

Comparison of the relative frequency of rheumatic manifestations in the aunts and uncles of rheumatic index cases on the paternal and maternal sides according to the history of rheumatic manifestations in the parents of whom they are siblings

Sibling of	Relation to index case	Paternal			Maternal		
		Number	Rheumatic		Number	Rheumatic	
			Number	Per cent		Number	Per cent
Rheumatic parents of index cases	Uncles	23	1	4.3	105	21	20.0
	Aunts	26	3	11.5	95	25	26.3
	Total	49	4	8.0	200	46	23.0
Non-rheumatic parents of index cases	Uncles	136	3	2.2	108	12	11.1
	Aunts	146	6	4.1	130	10	7.7
	Total	282	9	3.2	238	22	9.2

index cases, a greater proportion of the maternal than of the paternal aunts and uncles is found to be affected. The number of persons falling into each group in this table is not sufficiently large to allow significance to be attached to the individual percentages but they are consistent in that, in each instance they show a higher percentage of persons with rheumatic disease among the maternal aunts and uncles than among the corresponding relatives on the paternal side of the family.

This supplements the observation, brought out by Table III, that more rheumatic disease occurred on the maternal than on the paternal side of these families of rheumatic index cases and indicates that this difference is present whether the parents be rheumatic or non-rheumatic. This fact is worthy of consideration in any attempt to evaluate the hereditary factors in rheumatic disease.

SUMMARY AND CONCLUSIONS

Facts have been presented relative to the high incidence of rheumatic disease in the families of 96 rheumatic children. The percentage of persons with a rheumatic history, who had parents

with a similar history, was found to be consistent in two generations of these families and was 3.7 times as high as was found in a group of control families.

The offspring of the grandparents of the rheumatic and control index cases were studied to see if any relationship was present between the type of mating with respect to rheumatic disease and the percentage of children who were rheumatic. When one or both parents had a history of rheumatic manifestations a greater percentage of the offspring was rheumatic than was found in the offspring of parents who gave no history of rheumatic disease.

The percentage of female offspring of rheumatic mothers who had rheumatic manifestations was found to be almost twice as high as in the male offspring of these mothers.

A greater percentage of persons with rheumatic disease was found among the maternal aunts and uncles than was found among the paternal aunts and uncles of rheumatic index cases.

These findings suggest that hereditary constitution may play a role in the predisposition to this disease. The evidence here presented does not, however, exclude the possibility that infection plays an important role and that exposure may be the predominating factor.

BIBLIOGRAPHY

1. Read, Frances E. M., Giocco A., and Taussig H. B., The frequency of rheumatic manifestations among the siblings parents uncles, aunts and grand parents of rheumatic and control patients. *Am. J. Hyg.* 1938 27 719
2. Draper George, Studies in human constitution. IV Heredity and environment—the disease makers. *Am. J. M. Sc.*, 1926 171, 803
3. Irvine Jones, E. Acute rheumatism as a familial disease. *Am. J. Dis. Child.*, 1933 45 1184
4. Wilson M. G., and Schweitzer M. D., Rheumatic fever as a familial disease. Environment, communicability and heredity in their relation to the observed familial incidence of the disease. *J. Clin. Invest.*, 1937 16, 555

AUTOLOGOUS AND HOMOLOGOUS TRANSFUSION OF HUMAN ASCITIC FLUID

By H. A. DAVIS AND J. F. BLALOCK, JR.

(From the Department of Pathology University of Tennessee Memphis)

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The physiological availability of the individual intravenous solutions which have been used for experimental and clinical purposes varies. In secondary shock where an oligemia exists the value of protein-containing infusions is evident. Gum acacia saline solutions, used for the first time in human beings by Hurwitz (1) were administered extensively in traumatic shock during the World War (2). However, certain complications may follow the administration of gum-acacia solutions such as liver damage (3) interference with plasma protein regeneration (4) and a reduction in the oxygen content of the blood (5). This has affected to some extent its general popularity. Gelatin saline solutions have been recommended (6, 7) for the treatment of shock, but have fallen into disuse due to the fact that the gelatine leaves the blood stream very rapidly and, therefore, maintains the blood pressure for only a short period of time. Solutions of hemoglobin are capable of carrying oxygen and maintaining the osmotic pressure of the blood for a short period of time (8, 9). The hemoglobin is soon converted into methemoglobin resulting in renal damage during its excretion (10). However, hemoglobin in Ringer's solutions can maintain life in the mammal provided a minimum of intracellular hemoglobin remains in the blood stream of the experimental animal (8, 9). The hemoglobin in Ringer's solution for obvious reasons, has not been used in the human.

The first use of blood plasma and serum for transfusion purposes was made in the lower mammals by Guthrie and Pike (11). The hemococentration of the blood in various forms of shock provides a rational basis for the transfusion of blood plasma and blood serum. Excellent results have been reported following their administration in man (12, 13). The difficulty in obtaining blood from the usual sources has led to the use of blood from the placenta (14), the umbilical cord (15), and the cadaver (16).

The search for a blood substitute has induced us to determine the effectiveness of human pleural and peritoneal transudates (ascitic fluid). In a previous study upon the heterologous transfusion of ascitic fluid, it has been demonstrated to be of value as a substitute for blood in experimental shock in animals (17). In the present investigation, autologous and homologous transfusions of human ascitic fluid have been performed with special reference to their effects from the viewpoint of toxicity, transfusion reactions and effects upon blood pressure, pulse rate, and urine.

Properties of human ascitic fluid

Quantitative chemical examinations of a series of ascitic fluids revealed the following figures per 100 cc. of fluid: total protein, 20 to 31.9 grams; albumin, 0.96 to 1.9 grams; globulin, 0.6 to 0.8 gram; fibrinogen, 0.2 to 0.41 gram; nonprotein nitrogen, 18 to 40 mgm.; sodium chloride, 700 to 750 mgm., and calcium, 6.8 to 7.8 mgm. The albumin/globulin ratio ranged from 1.1 to 2.3. It will be seen that the protein content of ascitic fluid is approximately 28 per cent to 45 per cent that of blood plasma. It has been demonstrated that the osmotic pressure of the interstitial fluids of the body follows closely that of plasma of the blood (18) which suggests that the osmotic pressure of ascitic fluid is approximately that of blood plasma.

A prerequisite to the successful homologous and heterologous transfusion is the determination of the grouping of the fluid. This has been shown conclusively in animals (17). It has been pointed out that group specific agglutinins are present in peritoneal and pleural transudates (19, 20). Such substances are similar to those occurring in the blood. These facts render imperative the determination of the compatibility of the fluid with the blood of the prospective recipient. In support of the necessity for such determinations are the transfusion deaths resulting in several animals from the use of incompatible ascitic fluid. Ac-

cordingly, cross agglutination tests were performed throughout this investigation

Heterologous transfusion of ascitic fluid

A brief recapitulation of the results of transfusion of ascitic fluid in dogs may be pertinent at this point. It has been recognized previously that group specific substances are present in the lower animals, *e g*, in dogs (21, 22). By the performance of cross agglutination tests it was found that human ascitic fluid could be transfused, with safety, into dogs (17). No toxic effects were noted. Secondary shock was then produced by graded hemorrhage. The administration of the fluid was capable of raising the blood pressure from the shock level. The urine remained consistently free from albumin suggesting that the proteins of the fluid were utilized by the animals.

METHODS

In these studies both pleural and peritoneal transudates were available. However, in most instances ascitic fluid obtained from human patients suffering from portal cirrhosis of the liver or cardiac decompensation was used. The fluids were removed with aseptic precautions and stored in sterile flasks at a temperature of 0 to 5° C. No preservatives were used and bacteriological examinations were made at intervals using the blood-agar plate method. Prolonged refrigeration did not affect the physiological availability of human ascitic fluid (17). Each fluid was submitted to the following examinations: Kahn test, blood agar culture, and determination of the protein and electrolyte content. The Kahn and Wassermann tests were performed upon the blood of the donor of the fluid. Immediately before use, the fluid was filtered through six layers of fine gauze and heated to body temperature in a water bath.

A group of nine patients was selected to demonstrate the result of the autologous and homologous transfusion of human ascitic fluid. Cross agglutination tests (23) were carried out between the ascitic fluid and the blood of the prospective recipient which was diluted with 0.85 per cent sodium chloride solution. Two drops of ascitic fluid and one drop of diluted blood were used for each examination. In order to exclude the possible existence of an allergic sensitiveness to the fluid, an intradermal injection of 0.5 cc. of the ascitic fluid was made into the recipient. In none of the patients of the present series was the presence of such a sensitiveness noted. The fluid was administered intravenously by gravity at a rate of 10 cc. per minute. The usual aseptic precautions were taken. The amount of fluid which was administered varied from 500 to 1500 cc. All of these patients had been hospitalized for varying periods prior to the infusion so that a basal level of blood pressure and pulse rate had already been attained. The blood pressure and pulse rate

were determined before and during the introduction of the fluid. Urinary examinations were carried out before and after the transfusions.

Transfusion of ascitic fluid in humans

The autologous and homologous transfusion of ascitic fluid was studied with particular reference to the effect upon blood pressure (Table I). It will be noticed that the effect upon the systolic and the diastolic pressures varies. The systolic pressure remains unchanged or shows a depression of several mm Hg which is then followed by a slight elevation of blood pressure. In other instances, the systolic blood pressure did not alter. The diastolic pressure, however, showed an early increase of a few mm Hg and a slight depression later. On the other hand, an early depression of the diastolic blood pressure was seen occasionally. Examination of the blood pressure one hour after the completion of the transfusion revealed an approximate return to the pretransfusion level. These results suggest that the transfusion of ascitic fluid in individuals with relatively normal blood pressures, has only a slight effect in elevating the blood pressure. This is in accordance with the well known fact that infusions in individuals with normal blood pressures yield only a mild and temporary pressor effect. Later, the transfusion of ascitic fluid in one case of traumatic shock will be described.

TABLE I

Influence of homologous transfusion of human ascitic fluid upon blood pressure

Case	Blood pressure						1 hour later
	Before trans fusion	After transfusion					
		100 cc.	200 cc.	300 cc	400 cc	500 cc	
	mm Hg	mm Hg	mm Hg	mm Hg	mm Hg	mm Hg	
J B	142/100	142/96	142/112	142/110	144/96	152/110	150/108
J G	114/68	114/72	116/72	114/74	116/80	118/80	118/80
H R	124/70	124/70	122/76	122/74	122/74	122/72	122/72
M M	96/60	92/60	96/62	92/62	96/64	98/62	98/62
M L	132/66	130/62	126/62	128/56	130/58	130/58	132/60
W B	130/96	126/86	132/88	132/92	130/94	132/94	132/92
T W	140/48	148/52	146/50	142/58	142/56	142/58	158/60
J G	108/76	108/76	112/80	114/76	116/76	114/76	118/82
S S	136/82	134/72	138/74	136/76	134/76	136/76	150/84

The effect upon the pulse rate is also variable. In some patients there is present a slowing of the pulse, and in others an acceleration takes place. The reason for this is not clear. Exam-

ination of the urine revealed a transient cloudiness which persisted for several days following the transfusion. However, the usual tests for albumin were consistently negative. No evidences could be found of glycosuria or hematuria. These facts suggest that the ascitic fluid does not injure the kidneys and that the proteins of the fluid are utilized by the recipient.

The incidence of post transfusion reactions was low, only two occurring out of a total of ten transfusions. This low incidence is emphasized by the fact that the ascitic fluid which was used had been subjected to a prolonged refrigeration lasting from three to five months. Both reactions were of a mild character consisting of a brief rigor and moderate rise of temperature. No other untoward symptoms were observed. One of these patients was febrile prior to the transfusion and, therefore more susceptible to a reaction.

The question now arises as to the possibility of the occurrence of shock from the autologous retransfusion of ascitic fluid. It has been pointed out that homologous retransfusion shock can take place, and that, in dogs this is of an anaphylactic nature (24). In one patient, J. B. a second transfusion of his own ascitic fluid ten days after

the primary transfusion produced no evidences of shock. Evidently this patient had not developed any allergic sensitiveness to his own protein.

Ascitic fluid transfusion in traumatic shock

The experimental evidence of the efficacy of ascitic fluid in treatment of hemorrhagic shock in animals (17) suggested its use in clinical secondary shock. One patient in this series, A. H. was admitted to the John Gaston Hospital in a severe state of secondary shock resulting from a gunshot wound of the abdomen. At the time he was seen by one of us (H. A. D.), he had been in shock for a period of approximately 12 hours. The usual evidences of grave secondary shock were present—profuse sweating, restlessness, rapid feeble pulse, hypotension, slow sighing respirations, and semiconsciousness. Previous treatment which had included two infusions of isotonic glucose solution had proved ineffective in raising the blood pressure. Blood of the appropriate type was not available. Human ascitic fluid of the correct type was obtained and a transfusion of 1500 cc. of fluid was given. It will be noted (Figure 1) that the blood pressure which was 66/44 at the commencement of the transfusion rose gradually to 104/70. The objective

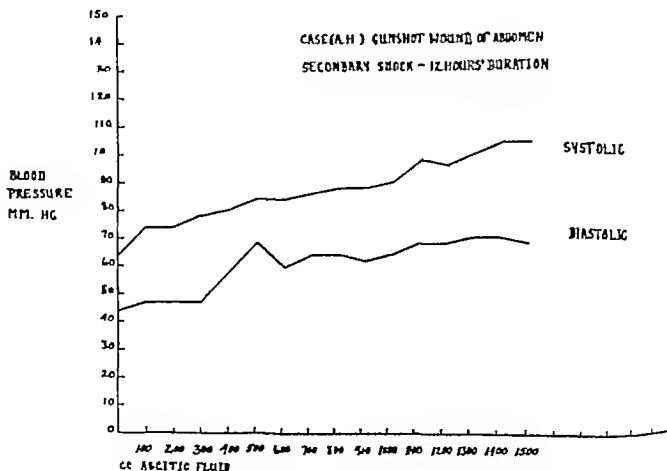


FIG. 1 INFLUENCE OF ASCITIC FLUID TRANSFUSION IN TRAUMATIC SHOCK

signs of shock diminished to a corresponding extent. At this time an exploratory laparotomy was performed. During the operation the systolic blood pressure fell to 70 mm Hg but a second transfusion of ascitic fluid raised it to 100 mm Hg. The patient finally succumbed 36 hours later. This case is of interest from two viewpoints. First, this patient received 2500 cc of ascitic fluid without any deleterious effects. Secondly, while the ascitic fluid elevated the blood pressure where previous infusions had failed to do so, this elevation of blood pressure was not maintained.

CASE REPORTS

J G male, white, 33 years. Diagnosis: lipid nephrosis of 3 years' duration. Two transfusions of ascitic fluid, one of 500 cc. and the other 1000 cc., no evidences of reaction.

T W male, white, 32 years. Diagnosis: chronic alcoholism of 10 years' duration. One transfusion of 500 cc. of ascitic fluid, no evidences of reaction.

W B male, white, 44 years. Diagnosis: tertian malaria of 3 weeks' duration. One transfusion of 500 cc. of ascitic fluid. No evidences of reaction.

R M female, white, 31 years. Diagnosis: syphilis of central nervous system. One transfusion of 500 cc. of ascitic fluid. Mild rigor followed transfusion, and temperature rose from 94 to 102.4° F.

L B female, colored, 46 years. Diagnosis: tertiary syphilis. One transfusion of 500 cc. of ascitic fluid. No evidences of reaction.

M L female, colored, 36 years. Diagnosis: epidermoid carcinoma of cervix uteri of 1 year's duration. One transfusion of 500 cc. of ascitic fluid, mild rigor and rise of temperature to 102° F followed by rapid recovery.

J B male, colored, 55 years. Diagnosis: portal cirrhosis of liver with ascites. One transfusion of 1000 cc. of his own ascitic fluid. No reaction. Ten days later a second transfusion of 100 cc. of his own fluid was given without any evidences of reaction.

H M male, colored, 40 years. Diagnosis: gun-shot wound of abdomen. One transfusion of 500 cc. of ascitic fluid, no evidences of reaction.

A H male, colored, 41 years. Diagnosis: gun-shot wound of abdomen with severe secondary shock. Two transfusions of ascitic fluid, the first being 1500 cc. and the second, 1000 cc. The first transfusion raised the systolic blood pressure from 64 to 104 mm. Hg. The second transfusion raised the systolic blood pressure from 70 to 100 mm. Hg. Blood of appropriate type was not available, and the final outcome was death in deep shock 36 hours after admission to the hospital.

COMMENTS

In a survey of the results of ascitic fluid transfusion, it becomes apparent that this fluid is not

toxic in human beings provided that the proper precautions are taken. In only two of the entire group of transfusions did there occur reactions, and these could have been avoided probably by a more thorough filtration of the fluid. It is our belief that the cause of these reactions was particulate matter which had escaped filtration. Bacterial contamination as a source of the reactions can be excluded in view of the care taken to avoid this factor. Denaturation of the fluid proteins by prolonged refrigeration is an unlikely possibility, although it cannot be definitely excluded as yet. In all of the fluids a varying amount of fibrin clotting takes place. This spontaneous defibrination may render the fluid slightly toxic.

It might be pertinent, at this point, to review the question of vasotonins in the fluid. Such vasotonins have been described in whole blood, blood serum, and blood plasma (25, 26, 27). These vasotonins are of two types, one being vasoconstrictor in character, and the other vasodilator. The vasoconstrictor substances are both temporary and permanent in nature (28, 29, 30). The vasodilator substances are present in fresh serum and plasma but usually disappear after refrigeration. The dilator substances have been analyzed and, apparently, are proteins (25) and are related to adenosine phosphoric acid (31). It has been pointed out that these vasotropic substances are present in all of the body fluids, including ascitic fluid (32). For this reason, it is important to consider the possibility of such substances being the cause of certain of the reactions to ascitic fluid transfusion. The changes in pressure following the use of serum and plasma has been ascribed by many to the presence of particulate matter which can be removed by repeated filtration. However, this mechanical theory cannot be reconciled with the fact that ergotamine prevents the pressor effect of serum and plasma extracts (27, 33).

How adequate, from a physiological standpoint, are blood plasma and serum in the replacement of whole blood? The concentration of erythrocytes in traumatic shock and in shock following burns suggests that the major cause of the oligemia is the loss of the fluid portion rather than the cellular portion of the blood from the vascular system. Further proof of this lies in the fact that the transfusion of blood plasma and blood serum has proven successful in the secondary shock of ex-

permental animals (11, 34, 35, 36, 37, 38) and of human beings (2 12, 13)

For this reason, it would seem to be logical to assume that the transfusion of ascitic fluid might be of value in conditions of shock associated with a concentration of the red blood cells

The lower protein content of ascitic fluid as compared with that of blood presents a further problem for solution. The concentration of ascitic fluid and its preservation in a "lyophile" form is being carried out by the method described by Florsdorf and Mudd (39). It is proposed to study the effects of this "lyophile" form of ascitic fluid in secondary shock. Lyophile serum has been used with promising results in experimental shock (40) and in clinical shock (41). However, severe reactions may follow the use of concentrated serum in animals (42) and in human beings (40, 41)

CONCLUSION

The transfusion of human ascitic fluid in group-compatible animal and human recipients is practicable. Reactions are few and relatively mild and, probably, will be eliminated by more thorough filtration of the fluid. Prolonged refrigeration does not affect the physiological availability of ascitic fluid. It is suggested that the value of the fluid may be enhanced by concentration. The properties of the concentrated or lyophile form of ascitic fluid are being investigated

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BIBLIOGRAPHY

- Hurwitz, S. H., Intravenous injections of colloidal solutions of acacia in hemorrhage. Preliminary note. *J. A. M. A.*, 1917 68, 699
- Robertson, O. H., and Bock, A. V., Memorandum on blood volume after hemorrhage. Special Report Series Med. Res. Com., London, 1918 25 213
- Dick, M. W., Warweg, E. and Andersch, M., Acacia in treatment of nephrosis. *J. A. M. A.*, 1935 105 654
- Hall W. K., The effects of intravenous injections of acacia upon certain functions of the liver. *Am. J. Physiol. (Proc.)* 1938 123, 88.
- Christie A. Phatak, N. M., and Olney M. B., Effect of intravenous acacia on physico-chemical properties of the blood. *Proc. Soc. Exper. Biol. and Med.*, 1935 32 670
- Hogan J. J., The intravenous use of colloidal (gelatin) solutions in shock. *J. A. M. A.*, 1915 64 721.
- Clark, G. W. Effects of intravenous injections of a colloid (gelatin) upon rabbit sera. *J. Immunol.*, 1918, 3 147
- Ambersson, W. R., Mulder A. G., Steggerda, F. R., Flexner J., and Pankratz, D. S. Mammalian life without red blood corpuscles. *Science*, 1933 78, 106.
- Green, A. A., Studies in the physical chemistry of the proteins. X. The solubility of hemoglobin in solutions of chlorides and sulfates of varying concentration. *J. Biol. Chem.* 1932, 95 47
- Bayliss L. E., Kerridge, P. M. T., and Russell, D. S., The excretion of protein by the mammalian kidney. *J. Physiol.*, 1933 77, 386.
- Guthrie, C. C., and Pike, F. H., The relation of the activity of the excised mammalian heart to pressure in the coronary vessels and to its nutrition. *Am. J. Physiol.*, 1907 18, 14
- Rubet, C., Brodin P., and Saint-Girons F., Des injections de plasma sanguin (plasmotherapie) pour remplacer le sang total. *Compt. rend. Acad. sc.*, 1918 167, 618.
- Filatov, A., and Kartaiskij N., Die Transfusion von menschlichem Blutplasma als blutstillendes Mittel. *Zentrabl. f. Chir.*, 1935 62 441
- Malinowski M. S., Smurnova, L. G., Boyarsanova, M. S., and Tarzanova, V. G., Use of placental blood in transfusion. *Sovet. khir.*, 1934 7 179
- Novikova, L. A., and Farberova, R. S., Organization of collection and use of umbilical cord blood for transfusion. *Sovet. khir.*, 1936 11 794
- Judme, S., La transfusion du sang de cadavre à l'homme. Masson, Paris 1933.
- Davis H. A., and White, C. S. Human ascitic fluid as a blood substitute in experimental secondary shock. *Proc. Soc. Exper. Biol. and Med.*, 1938, 38, 462.
- Gilman, A., and Cowgill G. R., Osmotic relations between blood and body fluids. IV Pancreatic juice, bile and lymph. *Am. J. Physiol.*, 1933 104 476.
- Emile Weil P., and Isch Wall, P., Hemo-agglutinines des divers liquides organiques. *Compt. rend. Soc. de biol.*, 1923 88 173
- Yosida, K. I. Über die gruppenspezifischen Unterscheide der Transsudate, Exsudate, Sekrete, Exkrete, Organextrakte und Organzellen des Menschen und ihre rechtsmedizinischen Anwendungen. *Ztschr. f. d. ges. exper. Med.*, 1928, 63 331
- von Dungern, E. and Hirschfeld, L., Ueber gruppenspezifische Strukturen des Blutes. *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 1911 8, 526.
- Thomsen, O., and Kemp T., Blutgruppendifferenzierung bei Tieren. I. *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1930 67, 251

hand was rapid in comparison with the flow in the forearm, when the collecting pressure was applied the rate of increase in the volume of the forearm was greater than that caused by the arterial inflow to the tissues of the forearm. This was due to the trapping in the veins of the forearm of the blood returning from the hand. With the body cool and the forearm bath at 30° C, the blood returned from the hand slowly enough not to influence appreciably the measurement of the blood flow in the forearm. When the body was heated, however, the flow to the hand increased much more than that to the forearm and, if the circulation to the hand was not obstructed, the calculated values for the flow in the forearm were greater than the true values. When the water bath surrounding the forearm was maintained at 43° C the circulation in the forearm was so rapid that the venous return from the hand had little effect on the measurement of the forearm flow, regardless of whether or not the body was heated. In 5 experiments on 3 subjects at room temperatures from 20 to 28° C and with the forearm bath at 43° C, the forearm flows averaged 104, 149 and 88 cc per minute, respectively, after the circulation to the hand was occluded by inflating a cuff distal to the plethysmograph to 300 mm Hg the flows averaged 95, 147, and 87 cc, respectively. During the reactive hyperemia following arterial occlusion Grant and Pearson (5) found the forearm flow to be so rapid that the returning blood from the hand had no effect on it.

In the studies of the vasomotor reactions of the forearm and calf reported here, the circulation to the hand and the foot was always completely obstructed by suddenly inflating cuffs just distal to the plethysmographs to 300 mm Hg. From 30 seconds to 2 minutes were allowed for the base line to become level before readings were taken. Occluding cuffs were also used in studying the effects of epinephrine and pitressin when the temperature of the forearm bath was below 43° C, and in some instances when the water bath was at 43° C. They were usually applied when the blood flow was determined with the water bath at 32 to 37° C, when the temperature of the room was not high enough to cause vasodilatation in the hand or the foot they were not used. In the study of the effects of exercise and of ar-

terial occlusion in the forearm and calf the circulation of the hands and feet was always undisturbed.

OBSERVATIONS

Vasomotor reactions in the forearm and calf

The vasomotor responses in the forearm and the calf of 9 normal subjects were studied with the water bath at temperatures of from 8 to 43° C. The circulation distal to the plethysmographs was obliterated by inflating cuffs to a pressure of 300 mm Hg. In the 2 subjects who showed the greatest responses, repeated observations were made on different days and the temperature of both the room and the water bath was varied. The stimuli routinely used to elicit vasomotor responses were pinching the skin of the chest, noise, and a deep inspiration. In a few instances mental arithmetic and the sudden application of an ice cold towel to the chest were employed. The pinches always produced pain, the noises were unexpected and loud, in order to startle the subject if possible. Less intense stimuli usually gave little or no response. The forearm and calf responded to these stimuli in one of three ways: (1) with a decrease in volume, (2) with an increase in volume, or (3) with a biphasic reaction, *i.e.*, an increase followed by a decrease in volume.

Decrease in volume. Six of the 9 subjects exhibited a decrease in volume of the forearm following noise or pinch, 3 showed no change. Four showed a decrease in volume of the calf after noise or pinch, 5 showed no change (Figure 1-B). Five subjects showed a decrease in volume of both the calf and the forearm after a deep inspiration (Figure 1-A), and 3 showed no change. One was not tested. Two subjects showed no response to any stimulus in either the forearm or the calf. For reasons to be stated below, the decreases in volume of the parts are considered to be the result of active vasoconstriction and will be called constrictor responses.

The constrictor responses in the forearm and the calf paralleled the vasoconstrictor reactions which have been shown to occur in the hand and the foot following similar sensory stimuli. They had a similar latent period of from 3 to 9 seconds and likewise were abolished when complete vasoconstriction was caused by cold, and greatly diminished in intensity or abolished when the vessels

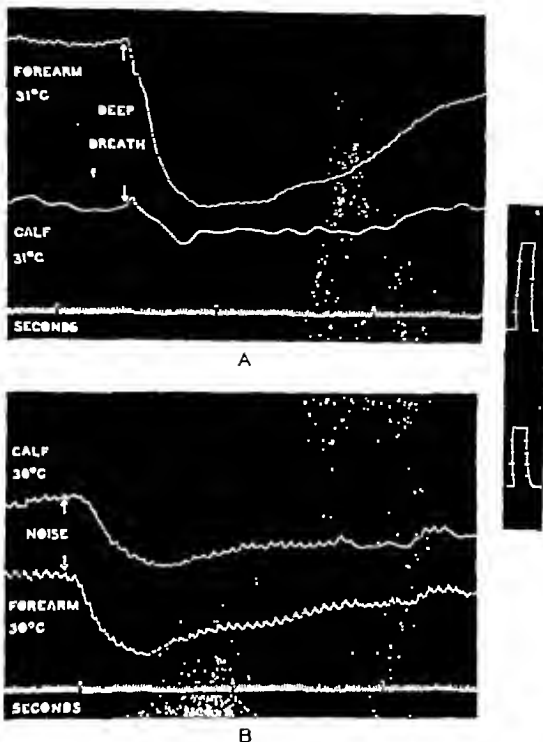


FIG. 1 VASOCONSTRICTION IN THE FOREARM AND CALF

In this and the following figures a rise in the base line indicates an increase, while a fall indicates a decrease in volume. The calibrations are in cubic centimeters: upper = forearm, lower = calf

A The effect of a deep breath. Room temperature 26° C.

B The effect of a sudden noise. Room temperature 32° C.

were sufficiently dilated by local heat. They usually lasted from 1 to 3 minutes but at times were of even longer duration. The intensity of the stimuli necessary to produce vasomotor responses in the forearm and the calf was greater than that required for the hand and the foot, and the magnitude of the response as measured in cubic centimeters per 100 cc. of tissue was usually less than in the hand and the foot.

As in the hand and the foot there was great variation in the degree of vasomotor response not only in different subjects but also in the same

subject on different days. The temperature range over which good constrictor responses were obtained in the forearm and the calf was however not so great as in the hand and the foot. The ideal temperature for obtaining the constrictor responses varied from subject to subject. In any one person both the environmental temperature and the temperature of the water bath influenced the state of the vessels. In general the responses were greatest with the room temperature between 30 and 33° C. and the temperature of the water bath between 30 and 37° C. Under these condi-

tions the subjects usually exhibited generalized vasodilatation. With the water bath at 43° C or above or at 20° C or below vasoconstrictor impulses were rarely obtained in response to noise or pinch though at times a deep breath was still effective. As a rule the vasoconstrictor responses in the forearm were greater, more easily obtained, and persisted over a greater range of temperature than those in the calf. Cold was as effective a stimulus as noise or pinch. Mental arithmetic produced less response.

It is well known that the efferent pathway for vasoconstrictor responses to sensory stimuli in the hand and the foot is through the sympathetic nerves supplying the vessels. As the vasoconstrictor responses in the forearm and the calf are similar to those in the hand and the foot, it is assumed that they also are transmitted by the sympathetic nerves. Since they are produced by such stimuli as noise and pinch, which do not cause a fall in blood pressure, the decrease in volume must be the result of active vasoconstriction in the vessels of the part enclosed within the plethysmograph.

Increase in volume. In addition to the constrictor responses, 4 of the 9 subjects showed at

some time during the experiments an increase in the volume of the forearm and calf following noise, pinch or the application of ice cold towels (Figure 2—A and B). For convenience any increase in volume regardless of the mechanism by which it is produced will be called a dilator response. These responses were obtained less frequently than the constrictor responses. They were obtained at times with the water bath at temperatures from 10 to 43° C. They were never produced by mild stimuli but occurred only when the patient had actual pain, was frightened by the noise or experienced actual discomfort from the ice cold towel. The stimuli employed were usually intense enough to produce quickening and deepening of the respiration and an increase in the force of the apex impulse. They frequently caused a rise in the blood pressure, but at times when an increase in volume was measured no increase in the blood pressure could be detected by the auscultatory method. The latent period between the application of the stimulus and the beginning of the increase in volume usually did not exceed 2 seconds and frequently was too short to measure by our apparatus. The duration of the dilator responses was usually from 15 to 30

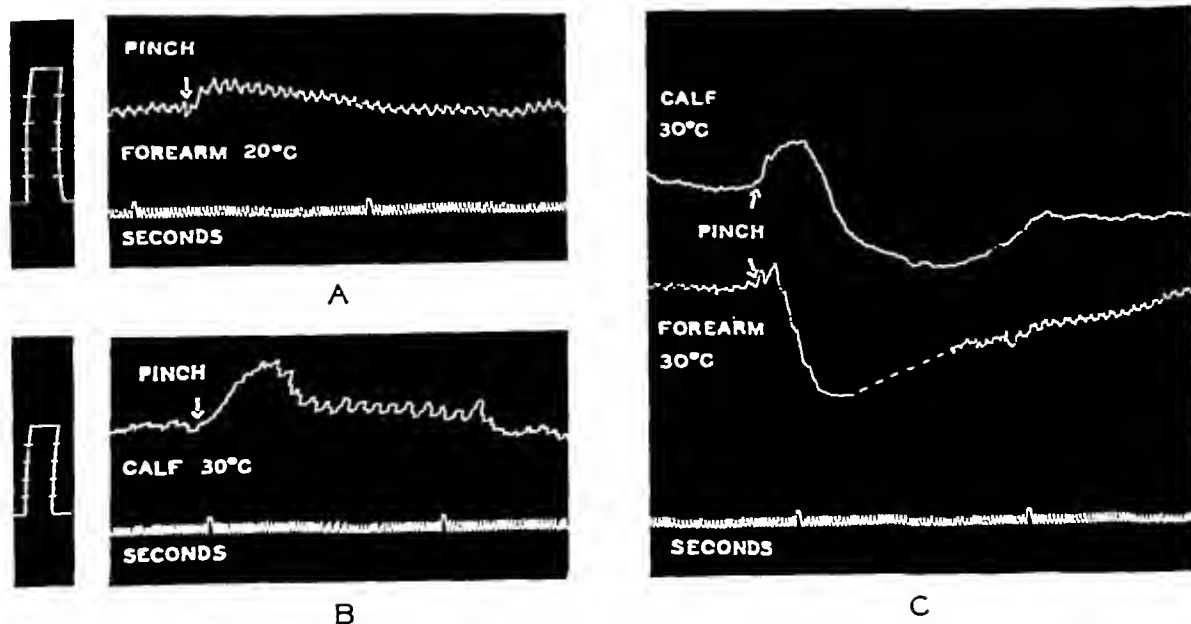


FIG. 2. DILATOR AND BIPHASE RESPONSES IN THE FOREARM AND CALF

A and B Dilatation induced by pinching the skin. Room temperature 1, 32° C. B 27° C.
C Dilatation followed by constriction in the calf produced by pinching the skin, vasoconstriction in the forearm from the same stimulus. Room temperature 32° C.

seconds in contrast to the constrictor response, which typically lasted from 1 to 3 minutes. Unlike the constrictor responses dilatation was more easily obtained early in the experiment, especially in the cases in which noise was the most effective stimulus. As the subjects became accustomed to the noise, its effect on the circulation diminished. Blood flow determinations made at the height of the dilator responses in the subject who showed the greatest increases in volume in the forearm and the calf showed no measurable change in the blood flow.

Biphasic reaction The third type of response was characterized by an increase in volume followed by a decrease (Figure 2-C). These biphasic responses were usually greatest with the water bath at or near 30°C . As in the monophasic reactions the latent period preceding the increase in volume was short.

The dilator responses observed following sensory stimulation can be explained in four ways. They may be due to (a) active reflex vasodilatation (b) a passive increase in the volume of the part as a result of vasoconstriction in the portion

of the extremity proximal to the plethysmograph (c) a passive increase in the volume of blood in the veins as a result of an increase in intrathoracic pressure (d) a slight increase in the volume of the vascular bed owing to a transient increase in cardiac output.

(a) When the increases in volume in the forearm and the calf were first observed it was assumed that they were the result of changes occurring in the muscles, since we had not observed similar responses in the hand and the foot which contain little muscle as compared with the forearm and the calf. In view of the fact that the stimuli used in the previous investigation had been weaker however the vasomotor responses in the hand and the foot were retested in the 2 subjects giving the greatest increase in volume in the forearm and the calf. The stimuli were of the same character and intensity as those used in the observations on the forearm and the calf. One subject showed on two different days an increase in volume followed by slight constriction in both the hand and the foot (Figure 3). In this case the water bath was cold enough nearly to obliterate

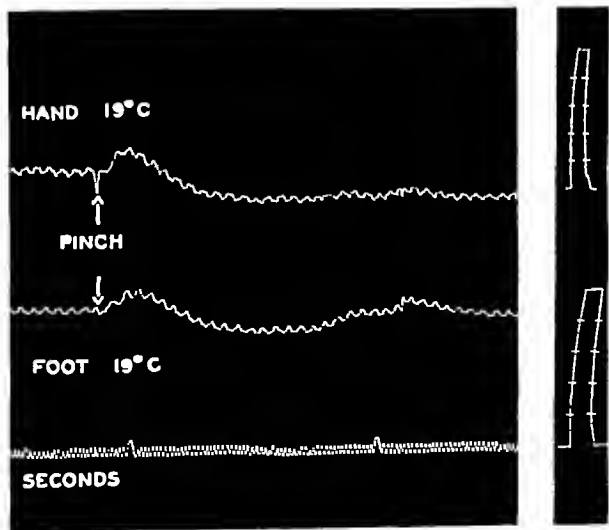


FIG 3 DILATATION FOLLOWED BY SLIGHT CONSTRICTION IN THE HAND AND FOOT
ROOM TEMPERATURE 23°C .

the constrictor response. The second subject showed the biphasic response only in the foot. Again, the latent periods preceding the increases in volume were short. This demonstration of an increase in volume in the hand and the foot, which contain little muscle, indicates that the similar response in the forearm and the calf is probably not the result of active vasodilatation occurring in the muscles of the forearm and the calf. Moreover, it is unlikely that the increase in volume is the result of active vasodilatation occurring in the skin, since the response in the forearm and the calf is obtained at times when the skin vessels are already greatly dilated by a local temperature of 43° C.

(b) It is possible that the increase in volume is the result of vasoconstriction in the portion of the extremity proximal to the plethysmograph. Regardless of whether the vessels inclosed in the plethysmograph are constricted by local cold or dilated by local heat, the vessels proximal to the plethysmograph may still be able to constrict. If the vessels inclosed within the plethysmograph did not change in caliber because they were either dilated by local heat or constricted by local cold, the effective blood pressure in these vessels might be raised as a result of vasoconstriction in other parts of the extremity. The short latent period preceding the increases in volume and the brief duration of the dilator responses as compared with the constrictor responses are against this explanation.

(c) Changes in intrathoracic pressure influence the volume of the parts inclosed in the plethysmographs, and produce the small respiratory waves frequently noted in the tracings (6). A slight expiratory effort against a closed glottis will produce an increase in intrathoracic pressure, which will cause the venous pressure to rise and increase the volume of the forearm and the calf. A few quick breaths also will at times produce an increase in the calf volume. Tracings of the increases in volume thus produced differ in two respects from tracings of increases resulting from stimuli such as pinch or noise. (1) When the breathing returns to normal the volume immediately returns to the base line, (2) the individual respiratory waves are larger.

(d) The dilator response to sensory stimuli is best explained as the result of a slight increase

in the volume of the vascular bed owing to a transient increase in cardiac output. This increase in cardiac output would not have to be great, as the maximum increase in volume was about 4 cc in a calf having a volume of 1800 cc, or about 0.22 cc per 100 cc of calf. It is true that by the auscultatory method the blood pressure did not always rise. It is possible, however, that the increase in pressure necessary to produce in an elastic system the volume changes observed may be very small. While no measurements of cardiac output have been made in the first few seconds following sensory stimulation an increase may well occur, as it has been shown that the latent period preceding the increase in pulse rate is short. The latent period preceding acceleration of the heart rate at the beginning of voluntary exercise is one cardiac cycle or less (7). Bainbridge (8), using anesthetized dogs, found that following stimulation of the central end of the sciatic nerve the heart rate increased after a latent period usually not exceeding 1 to 2 seconds. These authors (7, 8) showed that this initial acceleration of the heart rate was the result of a decrease in vagal tone and not the result of stimulation of the cardiac accelerator nerves. In 1 subject observed by us the effect of noise and pinch on the heart rate was studied with the electrocardiograph. After pinch the heart rate became more rapid for 3 to 4 beats, after noise the heart rate showed little change. This subject gave much better dilator responses to pinch than to noise.

If the increase in volume of the part resulted from increased cardiac output, the amount of increase would be influenced by both the size of the part and the degree of dilatation of the arterial bed before the stimulus is given. The greater amount of tissue present in the calf accounts for the ease with which dilator responses are obtained in the calf as compared with the forearm. The influence of the amount of dilatation of the vascular bed cannot be interpreted with such ease, as in 1 subject as large increases in volume were obtained at 10° as at 43° C.

Effect of local heat, arterial occlusion, and exercise on blood flow in the hand, forearm, foot, and calf in normal subjects

Local heat In subjects lying quietly in a comfortable position in a room at a temperature of

from 20 to 28° C and with the water bath at 32° C the values for the blood flow to the forearm and foot in 4 subjects and to the calf in 5 subjects each were at about the same level i.e. less than 3 cc. per minute per 100 cc. of tissue. The blood flow to the hand was somewhat greater, averaging 5 cc. in 8 persons. When the temperature of the water bath was raised the flow increased in all parts. At a room temperature of from 22 to 26° C with the water bath at 37° C the average values were hand (6 subjects) 10 cc. forearm (4 subjects) foot (4 subjects), and calf (2 subjects) around 5 cc. With the water bath at 43° C. the average flows increased to hand (18 subjects) 32 cc., foot (34 subjects) 17 cc. forearm (7 subjects) 14 cc., and calf (9 subjects) 9.5 cc. The blood flow at 43° C was little affected by variations in room temperature between 20 and 30° C. Table I gives the figures for the blood flow at 43° C in the hand, foot forearm and calf in 4 subjects.

TABLE I

Blood flow in the hand forearm foot and calf at 43° C recorded as cubic centimeters per minute per 100 cc of tissue

Subject	Hand		Forearm		Foot		Calf	
	Blood flow	Vol time	Blood flow	Vol time	Blood flow	Vol time	Blood flow	Vol time
E. S.	47	462	11	748	16	1175	5	1965
P. K.	29	534	11	870	16	1266	5	1920
H. F.	28	370	16	673	18	888	16	1430
E. A.	54	580	16	680	15	1260	10.9	1280

With the water bath at 32 to 37° C the blood flow in the hand and the foot was greatly increased by heating the body. No studies were made of the effect on the forearm and calf flows produced by heating the body. Grant and Pearson (5), however have shown that on moderate heating of the body there is little increase in the forearm and calf flows as compared with the increase in flow in the hand and foot. Recently Grant and Holling (9) have reported a considerable increase in forearm flow following intense heating of the body. We found that raising the local temperatures produced a definite progressive rise in the blood flow in the forearm and the calf.

The presence of arteriovenous anastomoses in the skin of the hand and foot (1) suggested that

the blood flow in the skin of the hand and the foot might be greater than that in the skin of the forearm and the calf. For the purpose of comparison the surface area of the respective parts was determined and the blood flow at 43° C. calculated as cubic centimeters per minute per 100 sq cm of skin (Table II). Thus the mass of

TABLE II

Blood flow in the hand forearm foot and calf at 43° C recorded as cubic centimeters per minute per 100 sq cm of surface area

Subject	Hand		Forearm		Foot		Calf	
	Blood flow	Skin area	Blood flow	Skin area	Blood flow	Skin area	Blood flow	Skin area
E. S.	45	482	23	393	28	678	15	656
P. K.	28	548	22	450	26	781	17	653

muscle present in the calf and the forearm was completely disregarded and the parts were treated as if the entire blood flow were to the skin. The flow in the hand calculated in this way was still greater than the flow in the other parts examined. The flow in the foot was also greater than that in the forearm and the calf.

Arterial occlusion. In order to determine to what extent the local heat of 43° C produced vasodilatation in the various parts, the beat stimulus was reinforced in the hand, forearm and foot in 2 subjects by a 5-minute period of arterial occlusion obtained by suddenly inflating cuffs proximal to the plethysmographs to a pressure of 300 mm. Hg. Blood flow determinations were made before and after the occlusion. In the foot the vessels were so completely dilated that in the period immediately following the arterial occlusion very little change in blood flow occurred. In the hand the flow increased between 25 and 50 per cent, but that in the forearm increased as much as 500 per cent. The difference between the response of the hand and foot may be due to the fact that the hand contains proportionally more muscle. However, the observed difference may be accounted for by the fact that the hydrostatic pressure is considerably greater in the foot than in the hand plethysmograph.

Exercise. Studies were made of the effect of exercise on the blood flow in the muscles of the forearm in 2 subjects and in the calf in 5 subjects. The exercise in the forearm consisted in

tightly closing and opening the fist at the rate of about 90 times per minute for 3 minutes, the muscles of the forearm being held as taut as possible. The exercise in the calf consisted of 3-minute periods of flexing and extending the ankle at the rate of about 60 times per minute. Flows were taken immediately after the period of exercise. The exercise in the forearm produced no constant change in the flow of the hand at temperatures of the water bath varying from 32 to 43° C, as it involved few of the muscles of the hand. However, it invariably produced a marked increase in the blood flow in the forearm. The actual increase in flow in the forearm was approximately the same (16 to 20 cc per 100 cc of tissue) regardless of whether the resting flow was 2 cc at 32° C or 10 cc at 43° C. In the calf the increases in blood flow after exercise were rarely as striking. In 5 subjects at 43° C the increase averaged 7 cc. per minute per 100 cc. In 1 subject tested at both temperatures the increase was the same at 32 and 43° C. The subjects were not able to keep the calf muscles taut during the period of exercise and this probably allowed better circulation through these muscles during exercise than occurred in the muscles of the forearm. The effect of 3-minute exercise of the toes was tried out in 1 subject at 30° C, and a rise in blood flow in the foot of only 1 cc. was obtained.

In organs which contain comparatively little muscle, such as the hand and the foot, external heat produces relatively complete dilatation and little further increase in blood flow can be induced by a 5-minute period of arterial occlusion. In the forearm, however, external heat causes great dilatation only in the vessels of the skin. Therefore, when the vessels of the muscle are dilated by arterial occlusion, the blood flow is greatly increased. Likewise, the fact that a given amount of exercise in the forearm and the calf produces approximately the same degree of increase in blood flow above the resting level at temperatures of from 32 to 43° C is evidence that the major portion of the vasodilatation resulting from the heat occurs in the skin.

Effect of epinephrine and pitressin on blood flow in the hand, forearm, foot, and calf

Epinephrine Epinephrine hydrochloride, 1 cc. of a 1/1000 solution, was injected subcutaneously

In the amounts used, epinephrine always caused a well marked increase in the pulse rate, systolic pressure and pulse pressure, and a fall in the diastolic pressure. The subjects became pale and complained of palpitation. At 43° C marked vasoconstriction occurred in the hand and foot, usually lasting for 2 hours. In 4 subjects the average flow in the hand fell from 38 to 15 cc per minute per 100 cc of tissue following the administration of epinephrine, the average flow in the foot in 2 subjects fell from 14 to 6 cc.

In the forearm and the calf the flow never decreased, usually it showed a definite increase coincident with the marked increase in the amplitude of the pulse waves (Figure 4). A slight increase in flow persisted after the pulse rate and the blood pressure had returned to the resting level. The greatest increase in blood flow usually occurred at the height of the rise in pulse rate and systolic blood pressure. In 8 experiments on 5 subjects at 43° C the average maximal increase in forearm flow was 4 cc, an increase of 40 per cent. As the average forearm volume was 751 cc, this was an increase of 30 cc per minute in the flow to the mass of forearm inclosed. In 1 subject at 31° C the forearm flow increased from 2 to 7 cc, an increase in total flow of 43 cc per minute. In another subject at 20° C the flow increased from 1.5 to 3 cc, or an increase in total flow of 13 cc per minute.

The calf was studied in only 2 subjects at 43° C, in one the flow showed no change and in the other the flow increased from 9 to 11 cc. As the calf volume was 1400 cc this represented a rise of 28 cc per minute in the blood flow to the part of the calf inclosed in the plethysmograph. In 2 subjects at 31° C the average calf flow increased from 1 to 3.5 cc. This represented an increase of 50 cc per minute to the portion of the calf within the plethysmograph (Figure 5).

Blood flow determinations were made before and after exercise of the forearm muscles in order to ascertain whether epinephrine altered the ability of these vessels to dilate. Three-minute periods of exercise were carried out in the forearm as previously described with the water bath at 43° C. Epinephrine, 1 cc of 1/1000 solution, was then injected subcutaneously and similar periods of exercise performed while the vasoconstriction in the hand and the foot was maximal. The ves-

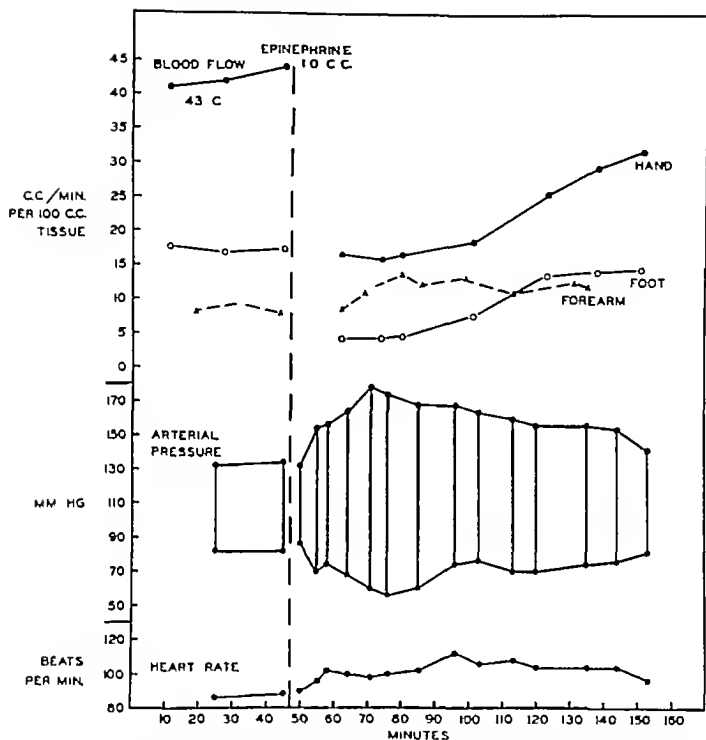


FIG 4 EFFECT OF EPINEPHRINE, 1 CC. OF 1/1000 SOLUTION SUBCUTANEOUSLY ON BLOOD FLOW IN THE HAND FOREARM AND FOOT IN THE SAME SUBJECT AT 43° C.

Determinations in the hand and foot were made simultaneously measurements in the forearm were taken on another day

sels dilated as fully with exercise as they did before injection of epinephrine. Since the exercise was difficult to quantitate the blood flow was determined in the forearm at 43° C in the period of reactive hyperemia following 3 minute arterial occlusion before and after the subcutaneous injection of 1 cc of 1/1000 solution of epinephrine. The resting flow of the forearm was increased by the epinephrine the response to arterial occlusion was not altered.

Pitressin Observations were made on the effects of 1 cc. of pitressin injected intramuscularly on the blood flow the blood pressure and the pulse

rate. Pitressin produced very little change in the pulse rate or the blood pressure the characteristic effect being no change in the systolic and a slight elevation of the diastolic blood pressure. All the subjects became pale and usually complained of abdominal cramps. In 6 experiments on 4 subjects with the water bath at 43° C injection of pitressin produced a decrease in the average blood flow in the hand from 43 to 15 cc. in 2 subjects the average foot flow dropped from 16 to 6 cc. In 6 experiments on 4 subjects the average flow in the forearm decreased from 13 to 10 cc at the height of the pitressin effect in 1 case, however,

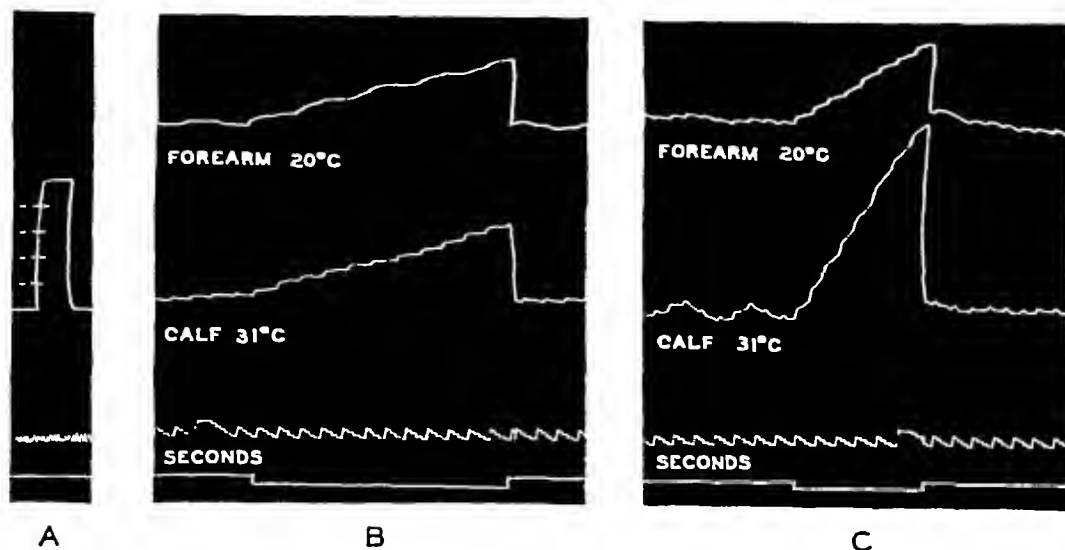


FIG 5 SIMULTANEOUS TRACINGS SHOWING EFFECT OF EPINEPHRINE, 1 CC OF 1/1000 SOLUTION SUBCUTANEOUSLY, ON BLOOD FLOW IN THE FOREARM AT 20° C AND IN THE CALF AT 31° C

A Calibration in cubic centimeters, upper = forearm, lower = calf

B Flow tracings before epinephrine

C Flow tracings after epinephrine Forearm volume = 850 cc, calf 2025 cc

no change occurred. In the calf in 1 subject at 43° C the flow decreased from 11 to 5 cc. Studies were made at 43° C on the response of the vessels of the forearm muscles to 3-minute periods of exercise, before and after injection of pitressin. After pitressin the resting forearm flow fell slightly, but after exercise it increased above the base line the same as before pitressin. In spite of the drop in the forearm flow the vessels of the muscles were able to dilate normally.

DISCUSSION

From these observations, the vasomotor reactions in the forearm and the calf appear to be qualitatively identical with those present in the hand and the foot. The constrictor response described is mainly or entirely the result of changes in the vessels of the skin of the forearm and the calf. The sensitivity of individuals to vasomotor stimuli varies considerably. We have observed persons in whom powerful vasoconstriction developed even in the presence of vasodilatation induced by local heat of 43° C. Grant and Pearson (5) state that they obtained either an increase or no change in volume in the forearm and the calf following sensory stimulation and that in no instance did they elicit vasoconstrictor responses.

In their illustration of increase in volume following a sudden noise the room temperature was 13° and the temperature of the water bath 30° C. Since our best constrictor responses were obtained in a warm room, the difference in room temperature may account for their failure to obtain constrictor responses. While our data suggest that the dilator responses are not the result of active vasodilatation, the responses in the sympathectomized forearm and calf must be studied before final conclusions can be drawn. Such a study will require several subjects since under the conditions of our experiments several normal persons give no dilator responses.

In the majority of the subjects the vasomotor responses were more easily obtained in the forearm than in the calf. When vasoconstriction was produced in both organs the change in volume was usually greater in the forearm than in the calf. Likewise, as shown by the greater blood flow at 43° C, the vessels of the forearm were able to dilate more widely than those of the calf, and the vessels of the hand more widely than those of the foot. This observation that the response of the vessels of the forearm to various stimuli is greater than the response in the calf is in accord with the observations of other ob-

servers that the blood vessels of the upper part of the body are more sensitive to both chemical and nervous stimuli than those of the lower extremities. It has been shown that the flushing of the skin produced by chemical agents, such as histamine (10) and acetylcholine (11) is greater in the upper part of the body. Likewise Ellis and Weiss (12) in a study of the vasomotor reactions in subjects with hemiplegia demonstrated that the disturbances of the vasomotor system pro-

duced by the lesions in the central nervous system were much more marked in the upper than in the lower extremity. They showed that edema occurred frequently in the paralyzed upper extremity, but only rarely in the paralyzed lower extremity and that it was the result of decreased peripheral resistance due to arteriolar dilatation. Thus arteriolar dilatation was much more marked in the paralyzed upper than in the paralyzed lower extremity.

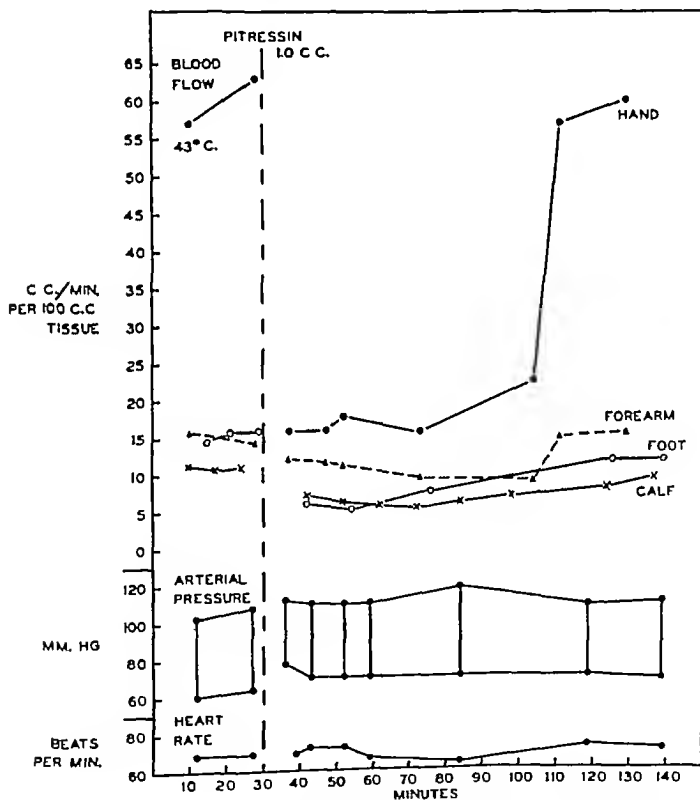


FIG. 6. EFFECT OF PITRESSIN 1 CC. INTRAMUSCULARLY ON BLOOD FLOW IN THE HAND FOREARM FOOT AND CALF AT 43° C.

Determinations on the hand and forearm were made simultaneously the calf and foot measurements were from another subject.

The observations reported show clearly that the peripheral blood flow in the extremities varies with the function of the tissue and that a stimulus that is very effective in increasing the blood flow in one tissue may cause relatively little response in another. The blood flow in the skin, particularly in the skin of the hands and feet, is regulated to a great degree by the need of the body to dissipate heat, and by heating the body the blood flow can be increased many times above that required for metabolism of the tissues. The blood flow in the muscles, however, is not directly concerned with heat dissipation and is not greatly increased even by local heat. Thus, heating the body or a part of the body appears primarily to drive the blood to the skin, especially to that of the hands and the feet. In contrast, exercise is a very effective stimulus for increasing the blood flow in the muscles, and the ability of the vessels of the muscles to dilate in response to exercise is little influenced by external heat ranging from 32 to 43° C.

The striking fall in blood flow in the hand and the foot after the subcutaneous administration of 1 cc of epinephrine is in marked contrast to the moderate rise in flow in the forearm and the calf. Since the vasomotor responses of the skin of the forearm and the calf seem to be in the same direction as the responses of the hand and the foot, it is logical to assume that the epinephrine causes some degree of constriction in the vessels of the skin of the forearm and the calf. If this is true, relatively more blood flows through the muscles than is indicated by the increase in total forearm flow. From these experiments it is impossible to say that the vessels of the muscles actually dilated as a result of the local action of the drug. The observed increases in flow might have been the result of the increased cardiac output forcing blood through vessels whose caliber had not changed. However, Grant and Pearson (5) state that epinephrine given intravenously in amounts small enough to cause only a transient rise in pulse rate and but a slight fall in systolic pressure produces vasodilatation in the forearm and the calf with an increase in the blood flow. Moreover, Weiss (13) has shown that the cardiac output remains elevated even after the pulse rate and the blood pressure have returned to normal, indicating that the blood flow in at least

some portions of the body must still be elevated. This vasodilatation may be the result of the increased production of lactic acid from muscle glycogen and of the increased basal metabolism induced by epinephrine, rather than a direct action of the drug on the vessels themselves. It has been shown that, in man, after the intravenous injection of small amounts of epinephrine the pulse rate and blood pressure return to normal much sooner than the blood lactic acid (14). A difference in the response of the vessels of the skin and muscles to body heating and to epinephrine has also been demonstrated by measurements of the local temperature. It has been shown by the use of thermocouples (15) that the application of heat to the upper extremities produces striking elevation in the surface temperature of the feet but has no effect on the temperature of the leg muscles, and that following spinal anesthesia the temperature of the muscles remains unchanged while the temperature of the surface of the skin becomes elevated. Following the injection of epinephrine a pronounced rise in muscle temperature with a striking fall in surface skin temperature was observed.

The effects of epinephrine, 1 cc of 1/1000 solution subcutaneously, and of pitressin, 1 cc intramuscularly, on the flow in the dilated hand and foot are quite similar, both cause a decrease in flow of about equal degree. Their effects on the circulation as a whole and on the blood flow in the muscles are quite different. Epinephrine causes a well marked increase in pulse rate, systolic pressure, pulse pressure, and minute output of the heart (16), the diastolic pressure is lowered. Pitressin, on the other hand, causes little change in pulse rate or blood pressure and no change in minute output (16). These differences are shown in the study of the peripheral blood flow in the forearm and the calf at 43° C, after epinephrine the flow increases and after pitressin it decreases. The vessels of the skin of the hand and the foot being equally sensitive to epinephrine and pitressin, it may be argued that the vessels of the skin of the forearm and the calf respond in the same way as those of the hand and the foot, though to a lesser degree, the decrease in the flow in the forearm and the calf at 43° C after pitressin being due to vasoconstriction in the skin and part of the increase in muscle flow after epine-

phrine being masked by a similar decrease in skin flow

SUMMARY AND CONCLUSIONS

1 Following strong sensory stimuli such as pinching the skin until pain is caused or a sudden loud noise, the forearm and calf respond in three ways (1) by a decrease in volume after a latent period of from 3 to 9 seconds (2) by an increase in volume after a latent period usually not exceeding 2 seconds (3) by a biphasic response with first an increase in volume with a short latent period usually not exceeding 2 seconds, and subsequently a decrease in volume.

2 Reasons are stated for the belief that the decrease in volume is the result of active reflex vasoconstriction, while the increase in volume is the result of a transient increase in cardiac output and a passive distension of the vascular bed

3 The sensitivity of vasomotor responses varies considerably in different persons. Variations in the same individual have been observed from time to time.

4 The vessels of the upper part of the body are more sensitive to physical, chemical and nervous stimuli than those of the lower part of the body

5 The blood flow induced by local heat of 43° C is greater in the hand and foot than in the forearm and calf even when the mass of muscle present in the forearm and calf is disregarded and when the blood flow is recorded as cubic centimeters per minute per 100 sq cm of surface area

6 Local heat of 43° C produces nearly complete vasodilatation in the skin, but only relatively slight vasodilatation in the underlying muscles

7 Exercise is a very effective stimulus for producing vasodilatation in the muscles but not in the skin. The ability of the muscle vessels to dilate in response to exercise is not appreciably influenced by varying the external temperature between 32 and 43° C.

8 Epinephrine 1 cc. of 1/1000 solution subcutaneously, causes a marked decrease in blood flow in the hand and the foot and a moderate increase in the blood flow in the forearm and the calf both at 32 and at 43° C

9 Pitressin, 1 cc. intramuscularly causes a great decrease in blood flow in the hand and the foot at 43° C as does epinephrine. At 43° C

it causes a moderate decrease in flow in the forearm and calf

10 Neither epinephrine nor pitressin interferes with the dilatation of the muscle vessels in response to exercise or arterial occlusion

11 The observation of Grant and Pearson (5) that unless the circulation to the hand is completely obstructed the plethysmographic method of measuring blood flow to the forearm is not accurate under all conditions has been confirmed

This investigation was carried out with the technical assistance of Miss Sophia M. Simmons

BIBLIOGRAPHY

1. Grant, R. T., and Bland, E. F., Observations on arteriovenous anastomoses in human skin and in the bird's foot with special reference to the reaction to cold. *Heart*, 1929-31 15, 385.
2. Freeman, N. E., The effect of temperature on the rate of blood flow in the normal and in the sympathectomized hand. *Am. J. Physiol.*, 1935, 113 384
3. Stead, E. A., Jr., and Kunkel P., A plethysmographic method for the quantitative measurement of the blood flow in the foot. *J. Clin. Invest.*, 1938, 17 711
4. Lewis T., and Grant, R., Observations upon reactive hyperaemia in man. *Heart*, 1925 12, 73
5. Grant, R. T., and Pearson, R. S. B., The blood circulation in the human limb observations on the differences between the proximal and distal parts and remarks on the regulation of body temperature. *Clin. Sc.* 1938 3 119
6. Kunkel P., and Stead, E. A., Jr., Blood flow and vasomotor reactions in the foot in health, in arteriosclerosis and in thromboangiitis obliterans. *J. Clin. Invest.*, 1938, 17 715
7. Gasser H. S., and Meek, W. J., A study of the mechanisms by which muscular exercise produces acceleration of the heart. *Am. J. Physiol.*, 1914 34 48.
8. Bainbridge, F. A., On some cardiac reflexes. *J. Physiol.*, 1914 48, 332.
9. Grant, R. T. and Holling H. E., Further observations on the vascular responses of the human limb to body warming evidence for sympathetic vasodilator nerves in the normal subject. *Clin. Sc.*, 1938, 3, 273
10. Weiss S., Robb G. P., and Ellis L. B., The systemic effects of histamine in man, with special reference to the responses of the cardiovascular system. *Arch. Int. Med.*, 1932, 49 360
11. Ellis L. B., and Weiss, S. A study of the cardiovascular responses in man to the intravenous and intra arterial injection of acetylcholine. *J. Pharmacol. and Exper. Therap.* 1932, 44 235

The body temperatures of the patients were determined by means of a suitable resistance thermometer placed in the rectum.

METHODS

Investigations were carried on in the special metabolic unit of the hospital. Sampling and analyses of food and excreta were performed as previously described (13), except for the following changes and additions. Stools were collected *individually* in tared glass containers, a suitable amount of distilled water was added, and the mixture weighed and agitated with a mechanical mixer until a uniform suspension was obtained. Aliquots were then weighed into pyrex dishes, dried on a steam bath, and ashed in a muffle furnace between 500 and 600° C.

Potassium was determined by first precipitating it from a solution of the ash as potassium sodium cobaltinitrite. This precipitate was then decomposed with strong hydrochloric acid and the potassium determined gravimetrically as chloroplatinate. Chlorine in urine, serum, and sweat was determined by the method of Van Slyke and Sendroy (14), that in food and stools according to the method of Birner (15). The sodium content of serum was found by ashing 2 or 3 ml of serum in a platinum dish. The ash was dissolved in dilute hydrochloric acid and transferred to a 50 ml. volumetric flask. Sodium free magnesia mixture was added to remove phosphates and the solution made to volume. The sodium was determined in an aliquot of the filtrate by the method of Barber and Kolthoff (14). Carbon dioxide content of the serum was determined by the method of Van Slyke and Neill (14). Serum solids were determined by weighing the serum in a covered weighing bottle, drying in an oven at 80° C., and dehydrating in a vacuum desiccator until weight was constant.

Diets

Patients F P and L H. received a liquid formula made of milk, eggs, and sugar, orange juice was given separately. Patients S B, and W D were given a formula made of powdered milk, lactose, sucrose, malted milk, and water. They also received graham crackers, tomato juice, and lemonade.

All liquid nourishments were made up in large quantities. Weighed aliquots were removed and saved for analysis while the remainder was kept in a refrigerator. Constant daily rations were weighed out on a torsion balance sensitive to 0.02 gram. The caloric and protein contents of the diets were estimated with due regard to size and age of the patients. In 3 of the patients, slight losses of weight occurred on these diets. In 2 of them, this loss lasted only for the first few days of the control periods.

Sodium chloride was given in the form of a solution measured with a volumetric pipette. After drinking this solution from a small glass, the latter was rinsed several times with distilled water and the patient drank each rinsing.

During the artificial fever, the diets, as well as salt were withheld from S B and W D. Instead, weak lemonade was given as tolerated. That taken by S B

during the 2 days of fever contained 443 grams of carbohydrate, that taken by W D contained 548 grams of carbohydrate. Patient S B also received 165 ml of whiskey.

Fluid intake was kept constant during the control periods. During fever it was increased to the amounts given in Table VIII.

Direct measurement of electrolytes lost through the skin

An attempt was made to measure the normal loss of electrolyte through the skin by having Patient L H spend 24 hours lying in a radiant energy fever cabinet which was kept just warm enough for comfort. A decrease in the urinary excretion of salt on the following day seemed to point to an unusually large secretion of sweat during the day spent in the cabinet. This finding, together with the restriction of normal activity and the considerable discomfort experienced by the subject, led us to abandon this procedure.

Another method was adopted in the case of Patients S B and W D. At the beginning of the day of observation, the subjects were washed with soap and water and then with distilled water. Pajamas and socks washed free of salt were worn for 24 hours. Activity was restricted to walking about the room. The maximum temperatures of the latter are recorded in Table V. Visible sweating was not present. At the end of 24 hours, the clothing was removed and later thoroughly extracted with distilled water in a continuous extractor until chloride free. The patients were again washed in distilled water. This bath water and the extracts of the clothing were concentrated and analysed. The amount of salt recovered was of the same order of magnitude (Table V) as that reported by others (16, 17). *Determination of the electrolyte content of the sweat during fever* was carried out in essentially the same manner. Each patient was washed with soap and water and thoroughly rinsed with distilled water. The mattress in the fever cabinet was covered with rubber sheeting and the pillow with oil silk. Both had been thoroughly scrubbed with distilled water. All cloths and towels used to wipe sweat off the face and head of the individuals had been previously extracted with distilled water, until the washings were chloride free. Sweat which gathered on the rubber sheet was siphoned into a bottle. After the fever treatment, the patient as well as the rubber sheet and pillow were washed with distilled water. The electrolytes were determined on the sweat which had been collected and the combined washings which had been evaporated to a small volume.

Indirect estimates of electrolytes lost through the skin

Indirect estimates of large losses of salt through the skin have been made by comparing the excretion of salt in the urine during control days with the urinary salt on days when sweating was excessive. The difference is presumably salt eliminated by the skin. Data published by Dill, Jones, Edwards, and Oberg (18) show that under these conditions the secretion by the sweat glands

can perhaps be measured with fair accuracy. An approximately correct average skin loss is, however, a difficult value to establish when sweating is at a minimum.

The apparent retentions of sodium, chloride and potassium as computed from analysis of diet, urine, and feces are ordinarily very small. Slight errors in the methods of analysis may be sufficient to cause the secretion of sweat to appear to be considerably greater or smaller than is actually the case. Moreover changes in the volume of body water or in its concentration of electrolytes may be enough to obscure loss through the skin. In spite of these handicaps indirect estimates of loss of salt in sweat were attempted in Patients S B and W D. Normal control days were selected.

In the first instance these included only a group of days on which changes in weight cancelled each other. The concentrations of sodium and chloride in the serum were at a nearly constant level and it was assumed that changes in electrolyte concentration in other body fluids would be at a minimum also. Positive sodium and chloride balances were taken as secretion from the skin.

Skin loss

Patient	Indirect estimate on days when weight changes cancelled			Found by direct determination on a single day	
	Days	Na	Cl	Na	Cl
S B	4-7	480 gram per day	735 gram per day	237 gram per day	217 gram per day
W D	3-8	330	520	69	83

The differences between direct determination and indirect estimate were considerable, but as the possibility of analytical errors existed in each method and as measurement of a single day's secretion from the skin could hardly be expected to give an average value, the result was not surprising.

In the second instance all of the control days, both before and after fever were included. Changes in weight and in nitrogen balance were taken into account, and corrections were made for the differences in volume of cell water and extracellular fluid using the nitrogen balance method of Gamble *et al.* (19). The apparent loss through the skin was smaller than by the first computation.

Skin loss

Patient	Indirect estimate with corrections for nitrogen balance and change of weight		
	Days	Na	Cl
S B	13	390	563
W D	13	79	254

Other combinations of control days and other methods were employed to make similar indirect estimates but

the 2 examples which have been cited illustrate the discrepancies which were encountered.

The most conservative values for skin losses were those found by the direct method. They have been employed without further attempt at justifying their use in computing balances in Patients S B and W D. Balances for F P and L H do not include skin losses on control days. The omission, however, does not seem to have had any important bearing on the validity of the data obtained during fever or in the period of recovery.

Clinical observations during fever

Patient F P, female, age 44. Induction of fever was begun at 8:50 a.m., June 19, 1935. After 80 minutes the temperature had reached 40.5 °C, where it was kept for 4 hours. The pulse rate was between 135 and 155 per minute during the treatment. The systolic blood pressure was 105 mm. Hg and the diastolic 80 mm. Hg at the beginning. It was not followed during the fever but the pulse remained of good quality. Her color was good and she perspired profusely during the entire treatment. Toward the end of the treatment she complained of headache, backache, and abdominal pain.

Patient L H, male, age 43. Induction of fever was begun at 9:50 a.m., April 18, 1935. The temperature reached 40.5 °C in 3 hours and was kept at that level for 4 hours without untoward effects. The pulse rate was between 125 and 135 per minute during the fever. The systolic blood pressure varied between 80 and 100 mm. Hg, the diastolic between 50 and 60 mm. Hg. Sweating was profuse during the period of induction, less obvious thereafter.

Patient S B, male, age 41. Induction of fever was begun at 9:00 a.m., August 12, 1936. In 70 minutes the temperature had reached 39.5 °C, where it was kept for 34 hours. During the first 4 hours his pulse rate was about 140 per minute. Thereafter it decreased gradually and remained between 100 and 120 per minute. The systolic blood pressure was 120 mm. Hg and the diastolic 70 mm. at the beginning of treatment. During the fever the systolic pressure varied between 88 and 115 mm. Hg and the diastolic between 50 and 70. After the first few hours the patient was somewhat restless and slept intermittently. His color was good at all times. Sweating was profuse during induction and the first 4 hours of the fever. After the sixth hour visible sweating ceased. Beginning with the eighth hour he complained of abdominal pain, localized about the umbilicus. This varied in intensity but gradually became more severe until fever was discontinued. After the twenty-fourth hour he complained of mild pain over the precordium. This was hard to evaluate because of the patient's apprehensiveness.

Patient W D, female, age 31. Induction of fever was begun at 8:30 a.m., August 24, 1936. After 100 minutes the temperature had reached 39.5 °C, where it was kept for 48 hours. The patient was cheerful, cooperative, and not in the least upset during the treatment. She slept a good part of the time. At all times sweating was much less than was observed in the other patients. Her color was good throughout. The systolic blood

TABLE I
Balance data on Patient F P (Hospital No 76562) June 16 to 23, 1935*

Day	Weight change	Sodium		Potassium		Chloride		Nitrogen	
		Intake	Balance	Intake	Balance	Intake	Balance	Intake	Balance
	grams	m.eq	m.eq	m.eq	m.eq	m.eq	m.eq	grams	grams
1	-460	95.6	-37.3	66.8	-26.2	98.9	-59.3	8.71	-1.51
2	-180	94.4	+15.3	75.0	+00.3	98.9	+8.7	8.92	-1.39
3	-30	95.6	+17.7	77.6	-11.2	98.9	+21.0	9.23	-1.77
4 { 6 a m -3 p m	-1370	47.9	-104.3	74.0	-5.7	48.7	-128.3	8.95	-0.64
3 p m -6 a m	+710	47.9	+46.9			48.7	+47.4		
5	+580	95.8	+91.1	71.3	+20.0	97.5	+88.0	8.93	-3.61
6	+50	95.8	+74.0	74.6	+1.2	97.5	+55.0	8.83	-1.64
7	+80	95.4	+32.1	74.6	-13.3	97.5	+24.5	8.76	-1.25

* Artificial fever 4th day. Induction time 80 minutes, temperature maintained at 40.5°C for 4 hours, recovery time 1 hour. Initial weight 1st day 54.560 kgm. Liquid diet 1300 calories.

pressure varied from 88 to 100 mm Hg, the diastolic from values too low to record to 70 mm Hg. The pulse rate varied between 115 and 135 per minute during the first 8 hours of fever, thereafter it gradually became somewhat slower and during the last 20 hours it was between 100 and 110 per minute.

PRESENTATION OF DATA

The condensed balance data are recorded in Tables I to IV. The fecal excretions of nitrogen, potassium, sodium, and chloride were included. The latter two were almost negligible.

The losses of electrolytes during the fever periods are summarized in Table V. Estimations are given of the fractions of total body electrolytes which these losses represent. It was assumed that extracellular water was equal to 20 per cent of the weight of the body at the beginning of fever and that sodium and chloride were confined to this compartment in concentrations which could be derived from analysis of serum. The percentage of water in serum was found directly in Patients F P and L H, in Patients S B and W D.

TABLE II
Balance data on Patient L H (Hospital No 100882) April 6 to 29, 1935*

Day	Weight change	Sodium		Potassium		Chloride		Nitrogen	
		Intake	Balance	Intake	Balance	Intake	Balance	Intake	Balance
	grams	m.eq	m.eq	m.eq	m.eq	m.eq	m.eq	grams	grams
1-10	0	111.7	+1.8	110.5	-1.1	117.7	+2.7	12.99	-0.15
11	-380	111.3	-39.5	109.7	-7.9	116.8	-9.5	12.67	-0.34
12	+410	111.3	+15.9	109.7	-9.6	116.8	+12.1	12.67	-0.34
13 { 7.30 a m -5 p m	-850	27.8	-141.3	109.7	-5.3	29.3	-102.0	12.67	+1.14
5 p m -7.30 a m	+550	83.5	+83.0			87.6	+87.1		
14	+440	111.3	+70.2	109.7	+13.7	116.8	+71.8	12.67	-2.09
15	0	111.3	+29.5	109.7	-2.8	116.8	+20.0	12.67	+0.16
16	-180	111.3	+5.1	109.7	-0.1	116.8	+4.7	12.67	+0.12
17	-20	111.3	-0.9	109.7	+3.3	116.8	-6.9	12.67	-0.50
18	-40	111.3	+1.2	109.7	-5.8	116.8	-2.6	12.67	+0.12
19	-100	111.3	+9.6	109.7	+10.6	116.8	+6.2	12.67	-0.50
20	+10	111.3	+5.7	109.7	+6.4	116.8	+5.8	12.67	+0.27
21	+60	111.3	+8.3	109.7	+2.1	116.8	+7.2	12.67	-0.16
22	-60	111.3	-8.9	109.7	+0.2	116.8	-6.5	12.67	-0.51
23	-140	111.3	-0.9	109.7	-15.1	116.8	-0.6	12.67	-0.81
24	-40	111.3	-0.1	109.7	-0.6	116.8	+0.6	12.67	-0.57

* Artificial fever 13th day. Induction time 3 hours. Temperature maintained at 40.5°C for 4 hours. Recovery time 1½ hours. Initial weight first day 53.150 kgm. Liquid diet 1875 calories. Skin loss included in calculation of balances on Days 11 and 13 only.

TABLE III

Balance data on Patient S B (Hospital No 89130)
August 5 to 20 1936 *

Day	Weight change	Sodium		Potassium		Chloride		Nitrogen	
		Intake	Balance	Intake	Balance	Intake	Balance	Intake	Balance
1	grams	mg	mg	mg	mg	mg	mg	grams	grams
2	-200	110.4	-63.0	113.0	+53.9	122.0	-53.3	10.33	-0.10
3	-810	110.4	-40.4	113.0	+54.9	122.0	-55.9	10.33	+1.11
4	-80	110.4	-12.5	113.0	+37.0	122.0	-15.9	10.33	+0.10
4-7 Daily average	0	110.4	+12.8	113.0	-7.2	122.0	+15.9	10.33	-0.43
8-9 Total	-2090	0.4	-350.3	20.8	-150.1	1.6	-239.1	0.0	-12.10
10	+530	110.4	+85.0	113.0	+41.0	122.0	+112.3	10.33	-1.97
11	-320	110.4	+77.8	113.0	+35.0	122.0	+111.4	10.33	-2.74
12	-250	110.4	+43.9	113.0	-8.3	122.0	+103.4	10.33	-3.87
13	-210	110.4	+53.7	113.0	-14.0	122.0	+92.4	10.33	-2.61
14	-190	110.4	+89.8	113.0	+10.7	122.0	+74.8	10.33	-0.29
15	-50	110.4	+11.6	113.0	-10.0	122.0	+10.0	10.33	-0.72

* Artificial fever—Days 8 and 9 Induction time 1 hour 10 minutes, temperature maintained at 39.5° C for 34 hours recovery time 1 hour 10 minutes Initial weight, 1st day 62.340 kgm. Liquid diet 2000 calories (Skin losses as recorded in Table V were included in the calculations for the balances for both the control and febrile days)

it was calculated from the serum protein concentration by use of the formula developed by Eisenman, Mackenzie, and Peters (21) To obtain the concentrations of sodium and chloride in extracellular water, the values for serum water were corrected for the Gibbs-Donnan effect (22) by multiplying by the factors 0.95 and 1/0.95 respectively Fifty per cent of the body weight was taken as the weight of intracellular water and the potassium concentration therein was considered to be approximately equal to the sodium in extracellular fluid The values for sodium potassium and chloride thus obtained were used in constructing Figures 1 and 2 which represent the daily changes in weight and electrolyte balances of Patients F P and S B

It will be noticed that losses of sodium and chloride through the skin varied from 65 to 19 per cent of the amount calculated to be in the extracellular fluid at the beginning of fever treatment (Table V)

It is well known that the ability to sweat varies considerably in different individuals Thus is illustrated by the electrolyte losses of these patients Patient W D was exposed to the same temperature as Patient S B but for 8 hours longer In spite of this the electrolyte loss of the former by way of the sweat was only a fraction of the loss of the latter This difference was reflected in the

clinical condition of the two patients Patient W D tolerated the fever in comfort while Patient S B became irritable and complained of pains in the legs abdomen and chest

Similar but less marked differences were found in the electrolyte losses of Patients L H and F P Because of more prolonged induction and recovery periods, the temperature of the former was elevated above normal for over 2 hours longer than that of Patient F P Nevertheless the loss through the skin was greater in the latter (Table V)

The amount of salt S B excreted in the urine was quite small, and previous experience leads us to believe that this must have been excreted during the first few hours Patient W D on the other hand excreted slightly more in the urine than was lost through the skin in spite of practically no intake The total loss of electrolyte from the body of these individuals was therefore, not dependent solely on skin loss (see Tables III, IV, and V)

The electrolyte intakes of Patients F P and L H. were kept the same during fever as on control days but were insufficient to offset the deficit of salt which developed because of sweating (Tables I and II)

Correlation between electrolyte and weight loss In each case except F P, the extracellular water

TABLE IV

Balance data on Patient W D (Hospital No 118263)
August 16 to 30 1936 *

Day	Weight change	Sodium		Potassium		Chloride		Nitrogen	
		Intake	Balance	Intake	Balance	Intake	Balance	Intake	Balance
1	grams	mg	mg	mg	mg	mg	mg	grams	grams
2	-210	43.3	-37.5	70.1	-3.1	48.3	-26.8	7.71	-1.04
3	-100	43.3	-7.1	70.1	+6.1	48.3	-0.4	7.71	-0.46
4	-100	43.3	-8.4	68.3	-10.8	47.9	-3.8	7.71	-1.73
5	+20	43.3	+6.8	68.3	+13.0	47.9	+0.4	7.71	-0.29
6	+180	43.3	+14.9	68.3	+2.7	47.9	+11.7	7.71	-1.31
7	+410	31.0	+70.8	68.3	-14.3	218.8	+82.3	7.71	-1.83
8	+410	31.0	-14.0	68.3	-21.4	218.8	-34.4	7.71	-1.06
9-10 Total	-900	1.0	-196.0	13.8	-58.6	8.3	-137.8	0.34	-11.61
11	+20	43.3	+34.9	68.3	+43.1	47.9	+31.7	7.71	+1.00
12	-90	43.3	-32.5	68.3	+7.3	47.9	-31.5	7.71	-2.24
13	-100	43.3	-23.8	68.3	-2.8	47.9	-23.8	7.71	-1.84
14	+330	31.0	+112.7	68.3	-1.0	218.8	+123.9	7.71	-1.19
15	-400	43.3	-58.0	68.3	+6.1	47.9	-40.0	7.71	-0.43

* Artificial fever—Days 9 and 10 Induction time 1 hour and 40 minutes temperature maintained at 37.5° C for 48 hours, recovery time 1 hour Initial weight 1st day 45.030 kgm Liquid diet 1550 calories (Skin losses as recorded in Table V were included in the calculations of the balances for both control and febrile days.)

TABLE V
Electrolytes during fever

Patient	Electrolyte	Control period		Fever period						
		Rectal temperature	Skin loss	Rectal temperature	Duration of fever	Skin loss		Net loss	Skin loss as per cent of amount in body	Net loss as per cent of amount in body
		° C	grams per day	° C	hours	grams	meq	meq		
F P	Sodium Chloride Potassium	36.5-37		40.5	4	2 992	130.0	104.3	8.0	7.0
						5 476	154.0	128.0	13.0	11.0
						0 361	9.3		<1.0	
L H	Sodium Chloride Potassium	36.5-37	0 234	40.5	4	2 510	109.1	141.3	7.0	8.5
			0 281			3 525	99.4	102.0	8.5	8.5
			0 373			0 687	17.6		<1.0	
S B	Sodium Chloride Potassium	36.5-37*	0 237	39.5	36	7 611	330.9	350.2	18.6	19.8
			0 217			9 438	265.9	289.1	19.0	22.0
			0 220			2 069	51.5	150.1	1.0	3.0
W D	Sodium Chloride Potassium	36.5-37†	0 069	39.5	48	1 914	83.0	168.0	6.6	13.4
			0 083			2 505	70.6	157.5	6.7	15.0
			0 123			1 141	29.2	68.6	<1.0	2.0

* Maximum room temperature Dry bulb 78° F Wet bulb 68° F

† Maximum room temperature Dry bulb 78° F Wet bulb 64° F

loss, if calculated from the losses of sodium or chloride and their concentration in the blood serum at the beginning of treatment, amounted to more than the loss of weight (Figure 1). If an estimate of the loss of potassium from intracellular water is included also, the expected loss of water is far in excess of the actual decrease in weight. In the case of S B, for example (Figure 1), the combined negative balances of sodium and potassium would account for a loss of 3 kilos, while only 2 kilos were lost. This seeming discrepancy is accounted for by the gradual dilution of the electrolyte remaining in the body (Table VII). In Patient F P the actual weight loss was greater than that calculated for the electrolyte loss, while the concentration of sodium and chloride in serum water increased slightly (Figure 2 and Table VII). The comparatively limited fluid intake of this patient appears to explain the difference in her response.

Several days were required to replace the deficit of sodium and chloride. In S B the retention lasted for 5 days. In each case, positive balances were greater than the amount lost during the days of fever (Figures 1 and 2). This may be at-

tributed to 1 of 3 causes, namely, analytical errors, greater electrolyte loss through the skin after fever than before, or increased storage after fever. Considerable experience with the methods which were employed leads us to conclude that analytical errors of this magnitude were unlikely. We have no direct evidence for or against either of the other possibilities. A labile vasomotor system after febrile illnesses is observed clinically. Manery *et al* have found connective tissue to be rich in sodium and chloride (23), it is possible that the increased mobility of joints after fever may have favored its retention there.

Changes in the blood and body fluids. The determinations of hematocrit, hemoglobin, serum proteins, and serum solids all point to moderate dehydration of the blood in 3 of the patients (Table VI). This was most pronounced in F P, and, as previously stated, seems to have been due to a rather low intake of water during the period when her secretion of sweat was at a maximum. In the fourth patient, W D, there was no evidence of hemoconcentration, probably because of a smaller and less rapid loss of water through the skin (Table VIII). In no case did

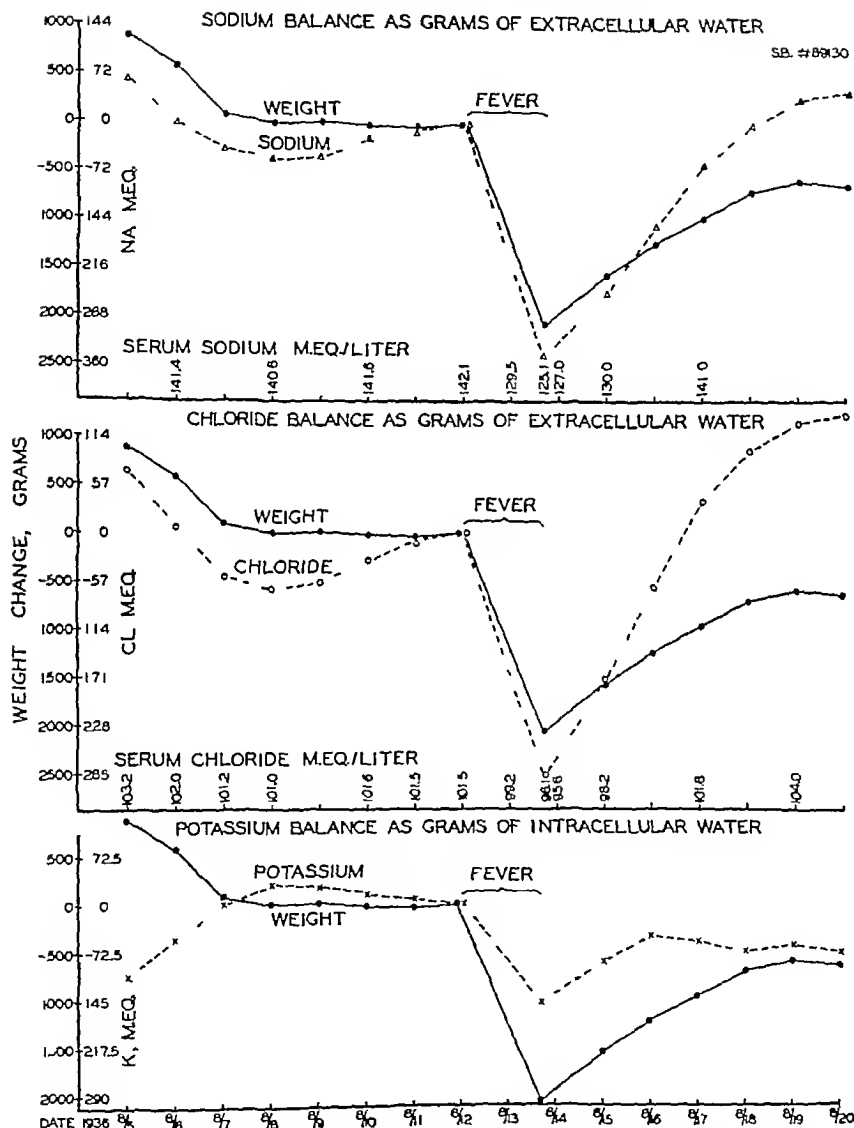


FIG. 1 WEIGHT AND ELECTROLYTE BALANCES IN PATIENT S. B.

Sodium and chloride are plotted to represent extracellular water, potassium to represent intracellular water

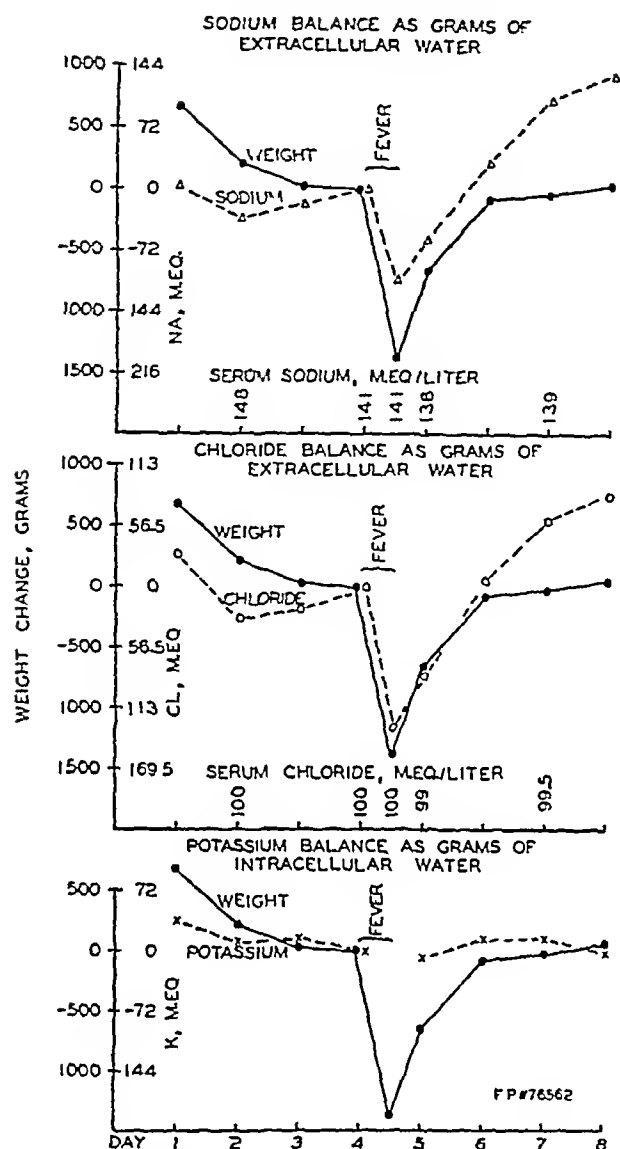


FIG 2. WEIGHT AND ELECTROLYTE BALANCES IN PATIENT F P

Sodium and chloride balances are plotted to represent extracellular water, potassium to represent intracellular water

the anhydremia approach that found by Talbott (3, 4) in studies of heat cramps

There were 3 instances in which the amount of sodium and chloride lost was sufficient to cause a definite reduction of the concentration of these substances in the blood serum (Tables VI and VII) The CO_2 content of the serum decreased also, but the decrease of sodium was greater in each case than the combined decreases of chloride and carbon dioxide This was to be expected

TABLE VI
Blood changes associated with artificial fever

Patient	Day	Remarks	Serum					Whole blood	
			Sodium	Chloride	CO_2 content	Solids	Protein	Hemoglobin	Hematocrit
			m.eq per liter	m.eq per liter	m.M per liter	per cent by weight	per cent	grams per 100 ml.	per cent
F P	2	Control	148.0	100.0	31.5	9.7		12.0	40.2
	4	Before fever	141.0	100.0	30.8	9.6		14.0	41.0
	5	After fever	141.0	100.0	29.5	11.3		14.0	45.0
	7		135.0	99.0	28.5	9.6		11.67	38.2
L H	3	Control		98.5	31.5			14.42	49.7
	7	Control		102.2	30.8	8.8	6.3	14.87	45.7
	10	Control		101.0	29.5	8.8	6.3	14.85	45.7
	13	Before fever		98.5	24.8	9.2	6.6	15.90	47.3
S B	1	Control	141.4	103.2	28.5				
	2	Control	141.0	102.0	30.4				
	4	Control	141.0	101.0	30.5				
	6	Control	141.6	101.6	30.7				
W D	7	Control	142.0	101.5	30.9				
	8-9	Before fever	142.1	101.5	30.7				
	10	During fever	129.5	99.2	25.3	6.44	7.14		
	11	After fever	125.1	96.1	24.9				
S B	10		127.0	95.6	27.9				
	11		130.0	98.2	28.5				
	13		141.0	101.8	28.1				
	15			104.0					
W D	2	Control	136.0	101.2					
	4	Control	135.0	104.8	25.1	6.70			
	9	Before fever	133.0	104.6	26.4	6.50			
	10	After fever	130.0	103.3	24.5	6.48			
S B	11		132.0	99.4	26.6				
	12		134.0	100.3					
	13		137.0	101.5					
	15		135.0	103.2	28.4				

TABLE VII
Changes in concentration of electrolytes in serum water associated with artificial fever

Patient	Remarks	Water in serum	Concentration in serum water		
			Na	Cl	CO_2
		per cent by volume	m.eq per liter	m.eq per liter	m.eq per liter
F P	Before fever	92.9	151.7	107.6	
	After fever	91.2	154.6	109.6	
L H	Before fever	93.8		106.5	31.4
	After fever	93.6		105.2	26.5
S B	Before fever	93.7	154.7	108.3	32.7
	After 24 hours of fever	93.2	138.9	106.4	27.1
	After 36 hours of fever	93.2	134.2	103.1	26.7
W D	Before fever	93.7	147.0	111.8	28.2
	After 24 hours of fever	93.7	139.0	110.4	26.2
	After 48 hours of fever	93.6	141.0	106.0	28.4

since the net losses of sodium were considerably in excess of the net losses of chloride (see fever days Table V)

Of the 2 subjects with mild symptoms resembling heat cramps, 1 (F P) concentrated serum

TABLE VIII
Losses of water through skin and lungs

Patient	Average normal day		Day or days of fever		
	Intake	Skin and lungs	Intake	Skin and lungs	Increase over normal day
	grams per day	grams per day	grams per day	grams per day	grams per day
F P	3550	860	3550	3340	2480
L H	3770	1135	4770	4385	3250
S B	3288	1180	4543	5085	3905
de la V	2816	882	4683	2423	1541

electrolytes in the other (S B) the loss of salt led to a decrease in concentration. A considerable disturbance of water balance occurred in each subject and was probably a more potent factor in the causation of symptoms than the change in concentration of electrolytes.

Composition of sweat The concentration of electrolytes in sweat is known to vary considerably (1). Wide variations in the proportions of different ions to one another as found in Table V are in accord with the work of McSwiney (24), who found the pH of sweat to vary from 5.1 to 7.76. Fishberg and Bierman (25) found large amounts of lactic acid in the sweat during hyperthermia. This may have been responsible for the excess of fixed base over chloride lost through the skin by 3 of our patients.

By using the data in Tables V and VIII and assuming a loss of 400 to 500 ml of water from the lungs one can derive an approximation of the average concentration of the sweat on the days of fever. The level of sodium ranged from 20 m eq per liter in W D to 45 m eq per liter in F P; the range of chloride was from 18 m eq in the former to 53 m eq in the latter. The concentration of potassium varied between 3 and 7 m eq per liter. Dill (1) has reviewed factors influencing the ability to form concentrated or dilute sweats. Among these is mentioned the possibility that a more dilute sweat is secreted when the level of salt in the blood falls below normal. The converse of this may have been true in F P, who was the only subject to increase the concentration of electrolyte in serum water during fever (Table VII).

Comment Although the conditions which led to the losses of variable amounts of water and electrolytes in the 4 subjects differed from one

another there was sufficient similarity between the duration and height of fever in F P and L H and in S B and W D to permit comparison and to bring out clearly the marked individual differences in their responses. The first two patients had short fevers and their net salt deficits were of the order of 10 per cent of the amount computed to have been present in the body at the beginning of treatment. Both tolerated the fever well. Net salt deficits in the 2 patients who were treated for longer periods were greater, but were hardly as large as one might have expected on the basis of the shorter fevers. Two explanations may account for the latter observation, (a) a lower body temperature was maintained during the longer fevers, (b) there was a fairly definite tendency for sweating to decrease when fever was prolonged beyond 3 or 4 hours.

There can be but little question that loss of extracellular fluid represented the chief and most important contribution from the body water. The reservoirs of extracellular water which were drawn upon are not known. Probably all of the tissues contributed water and salt, but it is unlikely that the quantities of fluid liberated were strictly proportional to the initial amount present in the tissue. Selective dehydration if it affected the blood plasma more than other tissues as appeared to be the case in some of Gibson's subjects might prove an embarrassment to the circulation. That selective dehydration may take place is apparent from the acute terminal experiments of Yannet and Darrow (26) who found that hyperthermia caused dehydration of the cells of the brains of cats. The reactions of this particular animal and man to high temperatures are hardly comparable. Man sweats profusely the cat almost none (27), so that the possibility of loss of water is much greater in the former. Other tissues beside the brain and blood must be affected. Of these the liver is certainly under suspicion for a low grade of jaundice is one of the complications of fever therapy (28).

Our experiments give little information with respect to the effect of hyperthermia on cell water. The potassium and nitrogen balances of the patients who were treated with 4-hour fevers and received their usual diets on the day of treatment were nearly the same as on control days. Appreciable losses of potassium and nitrogen were

encountered during the 36 and 48-hour fevers but were probably associated with catabolism of protein, as neither subject had even an approximately adequate caloric intake during these periods. In both the latter instances potassium seems to have been excreted without its full complement of cell water since sodium and chloride losses were nearly sufficient to account for the decrease in weight of the body. As with other electrolytes, loss of potassium was made good promptly on the days subsequent to fever.

Alkalosis has been reported in artificial fever (29, 30). According to Danielson *et al* (29), the pH of the serum was highest at the end of the period of induction and tended to fall somewhat as fever was prolonged. The alkalosis seemed to depend upon hyperventilation which brought about a primary carbon dioxide deficit. With hyperthermia lasting for 2 hours or more, there was a fall in the level of total base in serum and a decrease in BHCO_3 and BCl which together exceeded the decrease in base.

In our patients the electrolyte pattern of the blood serum reflected the net losses during fever. The sodium concentration of the serum decreased more than the sum of the decreases in bicarbonate and chloride and suggested a primary base deficit. It is possible, however, that increases in other cations may have offset the losses of sodium.

Proper preparation of the patient directed toward insuring normal hydration and electrolyte content of the body, and replacement of water and salt during the exposure to high temperature should, in a great measure, prevent development of symptoms of dehydration and electrolyte loss. Storage of extra salt before fever in healthy individuals probably can not be accomplished in appreciable amounts unless very large quantities are given or there has been previous depletion. Patient W D was given 20 grams of additional sodium chloride for 2 days before fever and retained about 33 grams (568 meq of Na and 578 meq of Cl), however, she had previously been on the relatively low salt intake of 25 grams per day. When Baird and Haldane (31) administered sodium chloride to healthy individuals in excess of their ability to excrete it (35 to 40 grams given during 2 hours) visible edema was produced and lasted for 8 to 24 hours. While no information is available concerning the extent

to which the plasma volume is increased during such expansion of extracellular fluid, the findings of McQuarrie *et al* (32) suggest that this may be quite large. These investigators found that when high salt feedings were continued several days marked elevations of blood pressure were produced. Such effects would be undesirable in patients with myocardial impairment.

The variations which characterize the individual patient's ability to excrete water and electrolytes make it difficult to state within fairly wide limits the individual's requirements during fever. Gibson and Kopp (8) have shown that the greatest shrinkage in the serum volume occurs during the rise and first hour of fever, and this is the period of most marked sweating. The magnitude of this deficit is probably influenced by the method of induction of fever, as well as the constitution of the patient (physical and mental status), previous food and water intake, individual differences in the activity of the sweat glands, and probably other factors. The losses through the skin are extensive enough in some cases to bring on a large deficit of salt and water. The kidneys then cease to produce urine. Other data (not included here) indicate that moderate sweating can continue during artificial fever only in the presence of adequate hydration of the body. Under such circumstances the kidneys continue to form urine. Cessation of sweating is a danger signal which should never be disregarded. Its significance as an indication of deficient fluid and sodium chloride is well illustrated in one of the reported cases (S B) who showed the greatest losses. His physical status appeared unsatisfactory during treatment. For this reason fever was stopped 12 hours sooner than had been the original intention. Patient W D, on the other hand, who had a smaller net loss of salt withstood a longer period of treatment in comparative comfort. Continued moderate sweating and the output of small quantities of urine every few hours seem to be good clinical guides and indicate the presence of adequate fluid and electrolytes.

SUMMARY

Electrolyte balances were measured before, during, and after artificial fever maintained at comparatively low levels (39.5° C and 40.5° C)

Skin losses were measured on a control day and during the fever

The skin losses of sodium and chloride during fever represented from 7 to 19 per cent and the net losses from 7 to 22 per cent of the amount estimated to be in the extracellular water at the beginning of treatment.

Differences in the losses through the skin were dependent largely upon variations in ability of the individual to sweat.

At the end of fever there was evidence of slight anhydremia.

Three patients lost more sodium than chloride, one patient more chloride than sodium in the sweat.

In 3 of the patients the concentration of sodium, chloride, and carbon dioxide of the serum water were decreased in one they were increased

The 2 patients showing the greatest losses of water developed symptoms which resembled heat cramps

Adequate storage beforehand, and replacement during treatment, particularly of sodium chloride and water, is necessary to prevent the development of symptoms of dehydration.

BIBLIOGRAPHY

- 1 Dill, D B., Life, Heat and Altitude. Harvard University Press Cambridge, 1938
- 2 Adolf E. F., Heat exchanges of man in the desert. *Am. J. Physiol.*, 1938, 123, 486.
- 3 Talbot J H., Heat cramps *Medicine*, 1935 14 323.
- 4 Talbot J H., Dill, D B., Edwards H. T. Stumme, E. H. and Consolazio W V The ill effects of heat upon workmen. *J. Indust. Hyg. and Toxicol.* 1937 19 258.
- 5 Moon, V H., Shock, its mechanism and pathology *Arch. Path.*, 1937 24 642.
- 6 Loeb R. F. Atchley D W., and Stahl, J., The role of sodium in adrenal insufficiency *J. A. M. A.*, 1935 104 2149
- 7 Kopp, I., and Solomon H. C., Shock syndrome in therapeutic hyperpyrexia. *Arch. Int. Med.*, 1937 60, 597
- 8 Gibson J G 2d, and Kopp I., Studies in the physiology of artificial fever I. Changes in the blood volume and water balance. *J. Clin. Invest.*, 1938 17, 219
- 9 Stecher R. M., and Solomon W M., The complications and hazards of fever therapy *Analysis of*

- 1000 consecutive fever treatments in the Kettering hyperthermia. *Ann. Int. Med.*, 1937 10, 1014
- 10 Ebaugh, F G., Barnack, C. H., and Ewalt, J R., Delirious episodes associated with artificial fever *Am. J. Psychiat.* 1936 93 191.
- 11 Wilbur E. L., and Stevens J B., Morbid anatomic changes following artificial fever with reports of autopsies *South. M. J.*, 1937 30 286
- 12 Hartman, F W., and Major R. C., Pathological changes resulting from accurately controlled artificial fever *Am. J. Clin. Path.*, 1935 5, 392.
- 13 Bassett, S H., Elden, C. A. and McCann W S., The mineral exchanges of man. I. Organization of metabolism ward and analytical methods. *J. Nutrition*, 1931 4, 235
- 14 Peters J P., and Van Slyke, D D., Quantitative Clinical Chemistry Vol. II Methods. Williams and Wilkins Co., Baltimore, 1932.
- 15 Birner M., Eine verbesserte Methode zur Chlorbestimmung in Organen und Nahrungsmitteln. *Ztschr. f. d. ges. exper. Med.* 1928 61 700.
- 16 McCance, R. A., Experimental sodium chloride deficiency in man. *Proc. Roy Soc. London*, A.B., 1936, 119 245
- 17 Freyberg R. H., and Grant, R. L., Loss of minerals through the skin of normal humans when sweating is avoided. *J. Clin. Invest.*, 1937 16, 729
- 18 Dill, D B. Jones, B. F., Edwards H. T., and Oberg S. A. Salt economy in extreme dry heat. *J. Biol. Chem.*, 1933 100, 755
- 19 Gamble, J L., Ross G S., and Tisdall, F F., The metabolism of fixed base during fasting *J. Biol. Chem.*, 1923 57 633
- 20 Bishop, F W. Lehman, E., and Warren, S L., A comparison of three electrical methods of producing artificial hyperthermia. *J. A. M. A.*, 1935 104, 910
- 21 Eisenman A. J. Mackenzie, L. B., and Peters J P., Protein and water of serum and cells of human blood, with a note on the measurement of red blood cell volume. *J. Biol. Chem.*, 1936 116 33
- 22 Peters J P., Body Water Thomas Springfield, 1935
- 23 Manery J F. Danielson I. S. and Hastings A. B., Connective tissue electrolytes. *J. Biol. Chem.* 1938 124 359
- 24 McSwiney B A., The composition of human perspiration. *Proc. Roy. Soc. Med.* 1934 27, 839
- 25 Fishberg E. H., and Bierman, W., Acid-base balance in sweat. *J. Biol. Chem.*, 1932 97 433
- 26 Yannet, H., and Darrow D C., The effect of hyperthermia on the distribution of water and electrolytes in brain, muscle and liver *J. Clin. Invest.*, 1938, 17, 87
- 27 Luciani, L. L. (transl. by Welby F. A.) Human Physiology Macmillan and Co London, 1913 Vol. 2, p. 486
- 28 Warren, S L., Chloride balance in artificial fever Abstracts and discussions of papers presented at

- the first International Conference on Fever Therapy, Hoeber, New York, 1937, p 34
- 29 Danielson, W H, Stecher, R. M, Muntwyler, E., and Myers, V C., The acid-base balance of the blood serum in hyperthermia. *Am. J Physiol*, 1938, 123, 550
- 30 Gibson, J, Kopp, I, and Pijoan, M, Acid-base balance during therapeutic fever Abstracts and Discussion of papers presented at First International Conference on Fever Therapy Hoeber, New York, 1937, p 33
- 31 Baird, M M, and Haldane, J B S, Salt and water elimination in man *J Physiol*, 1922, 56, 259
- 32 McQuarrie, I, Thompson, W H., and Anderson, J A, Effects of excessive ingestion of sodium and potassium salts on carbohydrate metabolism and blood pressure in diabetic children *J Nutrition*, 1936, 11, 77

CHANGES IN PULMONARY VOLUME FOLLOWING LOBECTOMY FOR BRONCHIECTASIS¹

By GUSTAF E. LINDSKOG

(From the Department of Surgery Yale University The School of Medicine New Haven)

(Received for publication December 12, 1938)

The ablation of any considerable portion of a vitally functioning organ immediately raises the question of a reduction in functional capacity. With respect to the removal of one or more lobes of the lung one may ask first, what are the immediate and remote effects on the total lung volume and its component parts, second, what changes occur in the hemorespiratory exchange, and how long do they persist?

Lilienthal (1) anticipated the first question in 1926 when he published reports on the vital capacity in 11 patients who had been subjected to partial lobectomy. Although he had no preoperative control observations he found, following operation, that the vital capacity ranged from 30 to 100 per cent of the theoretical minimal normal for the individual, and indicated that children showed a more facile return toward normal values.

Heuer and Andrus (2) were the first to report a study of hemorespiratory adjustments following experimental pneumonectomy. Dogs studied under resting conditions after removal of one entire lung showed an increase in alveolar CO_2 , lasting an average of 30 days, a slight increase in blood CO_2 , lasting 25 days, a fall in alveolar oxygen tension for 28 to 66 days and decrease in blood oxygen content up to the 11th day, returning to normal by the 30th day.

Longacre, Carter and Quill (3) have more recently modified and expanded these observations to include a study of the response to exercise in dogs before and after excision of the left lung. They have demonstrated that disturbances may persist for as long as five months (at least) after operation, as reflected in elevation of temperature and pulse rate, partial oxygen unsaturation of the arterial blood and diminished tolerance to anoxemia, these effects were present even though the measured subtidal lung volume in some animals had returned to the preoperative level dur-

ing this time. Derangements of this same type may occur with removal of one or two lobes but should be significantly less severe both as to degree or duration than when an entire lung is removed.

Our treatment of a group of patients with bronchiectasis and chronic lung abscess by lobectomy has afforded us the opportunity to study changes in the components of the lung volume picture by the determination of preoperative levels and then repetition of observations over long periods of time after operation. Five of our fourteen operative cases have seemed suitable for this study. The others were not utilized because of various undesirable preoperative situations, such as previous deforming operations on the chest wall, open bronchial fistulae, overwhelming cough and expectoration and too recent recovery from hemorrhage and pneumonia.

DEFINITIONS

Subtidal volume (functional residual) The air content of the respiratory tract at the conclusion of a normal (tidal) expiration.

Vital capacity The volume of air expired from the maximal inspiratory level to the maximal expiratory level.

Complementary air The volume of air inspired from the level of normal inspiration to the maximal inspiratory level.

Supplementary or reserve air The volume of air expired from the resting expiratory level to the maximal expiratory level.

Mean tidal air The average volume of air inspired or expired between the normal inspiratory and expiratory levels.

Total volume The air content of the respiratory tract at the maximal inspiratory level (that is the sum of the complementary, mean tidal and subtidal air).

METHODS

Patients were studied either in the fasting state or several hours after the last meal in the supine horizontal

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- the first International Conference on Fever Therapy, Hoeber, New York, 1937, p 34
- 29 Danielson, W H., Stecher, R. M, Muntwyler, E., and Myers, V C., The acid-base balance of the blood serum in hyperthermia. *Am. J Physiol*, 1938, 123, 550
- 30 Gibson, J, Kopp, I, and Pijoan, M, Acid-base balance during therapeutic fever Abstracts and Discussion of papers presented at First International Conference on Fever Therapy Hoeber, New York, 1937, p. 33
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Complementary air The volume of air inspired from the level of normal inspiration to the maximal inspiratory level.

Supplementary or reserve air The volume of air expired from the resting expiratory level to the maximal expiratory level.

Mean tidal air The average volume of air inspired or expired between the normal inspiratory and expiratory levels.

Total volume The air content of the respiratory tract at the maximal inspiratory level (that is the sum of the complementary, mean tidal, and subtidal air).

METHODS

Patients were studied either in the fasting state or several hours after the last meal in the supine horizontal

¹ Aided by a grant from the Fluid Research Funds of the Yale University School of Medicine.

position, the head resting on one pillow. All restraining clothing was removed and the arms laid parallel to the sides. The components of the vital capacity curve were determined in the well known manner with a Roth-Benedict recording spirometer. The subtidal volume was determined by the technic of Christie (4). This method depends on the dilution of alveolar nitrogen in the subject's lung by a controlled volume of oxygen rebreathed for five to seven minutes in a gas-tight spirometer system of calibrated dead space. A determination of the nitrogen percentage in the mixed spirometer gas sample at the end of a breathing period permits the calculation of the subtidal volume. The patients were studied preoperatively, and postoperatively after any existing bronchial fistulae had closed. The wound was usually healed and the pulmonary fields were clear by roentgenograms.

PROTOCOLS

A summary of the five cases is presented.

Case 1 Patient, W D, age 26, white male, Italian-American laborer (Table I)

TABLE I
*Case 1 (Patient W D) Bronchiectasis Right lower lobectomy (one stage) **

	Sub tidal volume	Vital capacity	Re serve air	Mean tidal air	Com ple-men-tary air	Total vol-ume
September 24, 1936	1650	1910	260	530	1120	3300
December 5, 1936	1910	1810	430	540	840	3290
September 24, 1937	1660	2230	410	510	1310	3480
December 22, 1937	1640	1970	360	580	1030	3250

* Date of lobectomy—October 8, 1936

Illness began in January, 1934, with cough, expectoration, chills and fever, subsiding in a few weeks so that he was able to return to work. Cough recurred, however, and in May, 1934, a roentgenogram showed an infiltration of the right lower lobe at its apex. Shortly, he was admitted to a sanatorium where he expectorated 250 to 275 cc. of purulent sputum daily, negative for acid-fast organisms on direct smear but occasionally positive on concentration. Bronchoscopic examination revealed an ulcerating, granulating lesion of the right lower lobe bronchus which was biopsied and a report of chronic non-tuberculous infection made. A right pneumothorax was begun in June, 1934. Despite the continued pneumothorax and a right temporary phrenic nerve paralysis performed in December, 1934, he continued to have daily fever, the expectoration of large quantities of foul-smelling sputum, chest pain, and hemoptyses until his admission to the New Haven Hospital on September 14, 1936. At that time six sputum examinations and one guinea pig inoculation were negative for acid-fast organisms. Another bronchoscopic examination revealed no specific pathology in the right lower lobe from which pus was constantly exuding. An injection of lipiodol into this

lobe showed a saccular dilatation of the medial bronchi in the posterior portion of the lobe and a collection of the lipiodol in two definite cavities. On October 8, 1936 under intratracheal cyclopropane anesthesia a densely adherent lower lobe was removed. The remaining cavity was drained by an intercostal catheter in the 9th space. He was discharged on the 61st postoperative day afebrile and free of sputum. At present, he appears to be in excellent condition, weighs between 180 to 190 lbs, has an excellent appetite, no cough or sputum except with an occasional head cold. Chest wound is well healed, and he has been working. Pathological diagnosis bronchiectasis, no evidence of tuberculosis.

Case 2 Patient, G H, age 16, white, male, American schoolboy (Table II)

TABLE II
*Case 2 (Patient G H) Bronchiectasis Left lower lobectomy (two stages) **

	Sub tidal volume	Vital capacity	Re serve air	Mean tidal air	Com ple-men-tary air	Total vol-ume
April 23, 1937	2410 ±10	1930	300	560	1070	4040
September 10, 1937	2900 ±80	2880	540	560	1780	5240
December 15, 1937	3210 ±120	3190	570	600	2020	5830
October 18, 1938	3910 ±110	3550	720	660	2170	6740

* Date of first stage May 11, 1937 Date of lobectomy (left lower) June 15, 1937

In August, 1936, while vacationing at the seashore he caught a cold and for about a week had a non-productive cough with pain in the mid-chest region. Following chills and fever, he was admitted to a hospital where a diagnosis of pneumonia was made. He began to have night sweats and gradually increasing amounts of sputum which became quite thick and evil-smelling. After four weeks in the hospital he went home but his sputum continued to increase and became more foul. Postural drainage and supportive measures did not improve his condition, and he lost 20 pounds in weight. Two paternal uncles died of pulmonary tuberculosis, but there was no contact with the patient.

He was admitted to the New Haven Hospital on November 19, 1936, acutely ill. Sputum output ran as high as 230 grams daily. His temperature and white count were elevated. A roentgen examination of the chest revealed atelectasis and pneumonia in the left lower lobe. The tuberculin test was negative to 0.1 mgm., and the sputum was negative for acid-fast organisms. A lipiodol examination revealed a saccular bronchiectasis of the left lower lobe and a normal right bronchial tree. After bronchoscopic treatment the patient began to improve. A left artificial pneumothorax was carried on for three months preparatory to an operative exploration, and the

patient was discharged home to convalesce. He returned in April 1937 after a fairly good winter with low-grade fever increasing sputum, and loss of six pounds in weight. On May 11 1937, a first stage lobectomy was done. The sputum output showed a remarkable immediate and permanent improvement. On June 15 five weeks after the first operation the wound was re-opened, and the lower lobe was easily excised. The convalescence was uncomplicated. The chest wound healed *per primam*. The drainage tube was removed from the chest on the 27th day. There was never clinical evidence of a bronchial fistula. A roentgenogram of the chest on July 14 showed a clearing of the left lung field with compensatory enlargement of the left upper lobe. The patient was discharged on the 32d postoperative day.

Sections of the excised lobe revealed many dilated, saccular thin walled bronchiectatic channels with but little involvement of the surrounding lung parenchyma.

The patient is now entirely symptom free and has returned to his high school classes taking part in all activities including gymnastics.

Case 3 Patient E. M., age 22, white, female, American (Table III)

TABLE III

Case 3 (Patient E. M.) Bronchiectasis Left lower lobectomy (two stage) *

	Subtidal vol ume	Vital capac- ity	Re- serve air	Mean tidal air	Com- plemen- tary air	Total vol- ume
May 12 1937	2970	2560	590	540	1430	4940
September 17 1937	3010	2480	780	540	1160	4710
December 8, 1937	2880	2660	720	560	1380	4820
April 19 1938	3250	2830	740	600	1490	5340
October 12 1938	3350	2800	700	570	1530	5650

* Date of first stage May 14 1937 Date of lobectomy July 2 1937

At the age of two the patient had infantile paralysis which left her with both legs flaccid. At ten she began to have nasal discharge, chronic cough, and sputum. At 12 she had whooping cough, following which her cough was always much worse and about three times a day she raised a half cupful of thick purulent malodorous sputum. The patient's father was said to have a chronic pulmonary lesion diagnosed as tuberculosis.

She was admitted to the New Haven Hospital on March 29 1937. She presented slight dullness and suppression of breath sounds at the left base posteriorly with fine moist râles. There was no clubbing or cyanosis of the finger tips. A lipiodol examination of the right and left lower lobes was carried out, and showed a bronchiectatic dilatation of the left lower lobe bronchi. Sputum was repeatedly negative for acid fast organisms. A series of eight bronchoscopic drainages was carried out without any improvement in the cough or amount of sputum which approximated 30 to 50 grams daily. On May 14 1937 under avertin and intratracheal cyclopro-

pene anesthesia a first stage operation was performed, the pleural surfaces being free of adhesions. The patient made a smooth convalescence and returned home to rest between stages. She returned on June 29 1937 following a very severe hemoptysis, approximating one cupful of fresh blood. On July 2, the left chest was re-opened through the healed wound with an additional resection of the 7th rib and the left lower lobe was removed. The chest was drained by means of an intercostal tube. The patient made an excellent convalescence. Evidence of a small bronchopleural fistula was present from about the 15th to the 21st day. Sputum was absent entirely after the 24th day and the patient was discharged home in six weeks.

She has gained in weight, has had no further sputum or blood-streaking and cough is only occasionally present when associated with a maxillary sinusitis for which she has since submitted to a submucous resection without any chest complications.

Case 4 Patient, G. R., age 16 white, Italian American male (Table IV)

TABLE IV

Case 4 (Patient G. R.) Bilateral bronchiectasis Bi-lobectomy (right middle and lower lobes) *

	Subtidal vol ume	Vital capac- ity	Re- serve air	Mean tidal air	Com- plemen- tary air	Total vol- ume
September 11 1937	2100 ±10	1160	210	460	490	3050
January 5 1938	2090 ±80	1360	380	460	520	3070
May 23 1938	1930 ±40	1730	450	480	800	3210
October 11 1938	2360 ±90	1910	490	520	900	3780

* Date of operation—September 14 1937

At the age of two years the patient had diphtheria followed by bilateral bronchopneumonia. He developed a right empyema which was treated by thoracotomy at another hospital. Following this illness the patient had a continual chronic cough with the production of scant, purulent sputum and was always under par physically. At the age of seven, roentgenograms of the chest were made and were reported to show fibrotic changes in both lower lung fields. At the age of twelve because of persistent cough and sputum, general malaise weakness and inferior general development the patient had lipiodol examination of the chest with a resultant diagnosis of bilateral bronchiectasis involving the lower lobes. At the age of thirteen he had a very severe hemoptysis and the tuberculin test which had previously been negative became positive. On July 13 1937 at the age of sixteen he was admitted to this hospital with a complaint of fever and pain in the left chest. Physical and roentgenographic examination revealed a pneumonia involving both lower lobes with fluid in the left chest. On the 3d

YEAST AS AN EXTRINSIC FACTOR IN RELATION TO PERNICIOUS ANEMIA

By R. W. HEINLE AND F. R. MILLER

(From the H. K. Cushing Laboratory of Experimental Medicine, Department of Medicine,
Western Reserve University and the Medical Service, Lakeside Hospital, Cleveland)

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Wintrobe (1) recently stated that certain patients with typical pernicious anemia could be thrown into complete remission by oral treatment with dried unautolyzed yeast, and he concluded that there was in the yeast some hematopoietic factor specific for the disease. As further argument for this belief he stated that the addition of normal human gastric juice did not increase the potency of yeast more than it increased the potency of liver extract. Other workers have previously reported successful treatment of pernicious anemia with preparations of autolyzed (2) and unautolyzed (3) yeast.

In this report we give our observations on 2 patients with typical Addisonian pernicious anemia in relapse, who were treated with yeast in the manner outlined by Wintrobe. The response ob-

tained was then compared with the response from material of known hematopoietic activity. Both patients showed absence of free hydrochloric acid after subcutaneous administration of 10 mgm. of histamine.

Patient C. W. was given ordinary brewer's yeast daily for 10 days (Figure 1). The attempt was made to administer 2 grams of yeast per kgm. of body weight, but the patient was not always able to take this amount. She never took less than 10 gram per kgm. of body weight, however, and consumed a daily average of 1.7 grams per kgm. of body weight over the 10-day period. During the test periods the diet contained no liver meat, or eggs. On the sixth day, the reticulocyte count rose to 20 per cent. After 10 days regular commercial ventriculin (Parke, Davis and Co.)

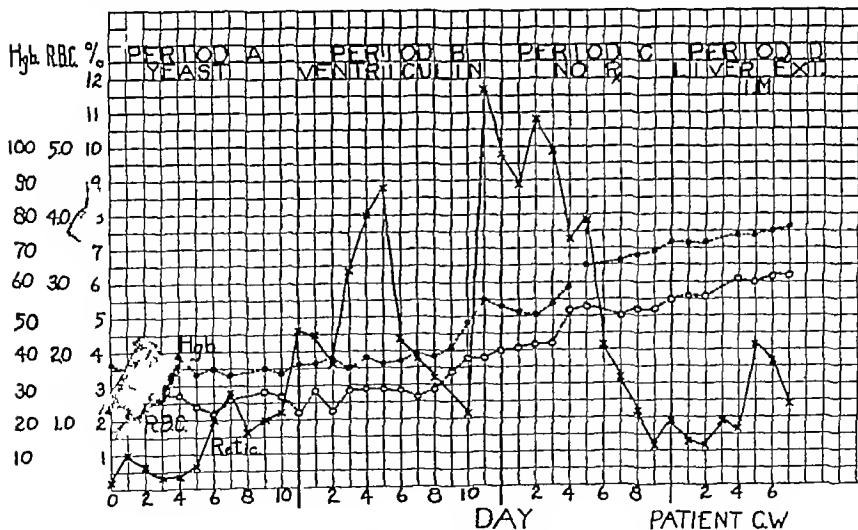


FIG. 1. PATIENT C. W. COMPARATIVE HEMATOPOIETIC RESPONSE TO YEAST, VENTRICULIN, AND PARENTERAL LIVER EXTRACT

was started, 15 grams daily (Period B, Figure 1). However, 14 days after starting yeast, the reticulocyte count rose to 8.8 per cent and the red blood corpuscle count and hemoglobin began to rise. Although this response occurred during administration of ventriculin, there can be no doubt that it was evoked by the yeast administered during the preceding period. This is proven by the fact that there was a subsidence of reticulocytosis followed by a second reticulocyte peak of 11.7 per cent on the tenth day after starting ventriculin. It is well known that two reticulocyte responses to continuous therapy with the same material in equal daily amounts do not occur (4). During the 10 days of Period C (Figure 1), no medication was given and the reticulocytosis subsided, although the red blood corpuscle count and hemoglobin continued to rise. In Period D (Figure 1), 5 cc of liver extract (Campolon, Winthrop) were administered intramuscularly daily. On the fifth day there was a reticulocyte response of 4.2 per cent. From this it is concluded that the yeast evoked a partial hematopoietic response which was not as great as that following 15 grams of ven-

triculo daily. Further, the ventriculin, in the amount given, was not as effective as the parenteral liver extract, as evidenced by the further reticulocyte rise after parenteral administration of liver extract.

Patient C H received ordinary brewer's yeast during the 15 days of Period A (Figure 2), in doses of 2 grams per kgm of body weight daily. He experienced no difficulty in taking the medication. He was on an ordinary house diet at the time, with the exception that liver was omitted, since it was desired to provide maximum opportunity for a response. The reticulocytes reached 5.9 per cent on the eighth day after starting yeast. During the next period (Period B, Figure 2) ventriculin was administered in doses of 15 grams daily. On the tenth day after starting ventriculin the reticulocyte count rose again to 5.2 per cent. In the last period, crude liver extract, 1 cc derived from 5 grams of liver (Solution of Liver Extract, Lilly), was administered intramuscularly in doses of 2 cc daily. On the eighth day of such treatment (Period C, Figure 2) the reticulocyte count reached 13.0 per cent and there was an increase in

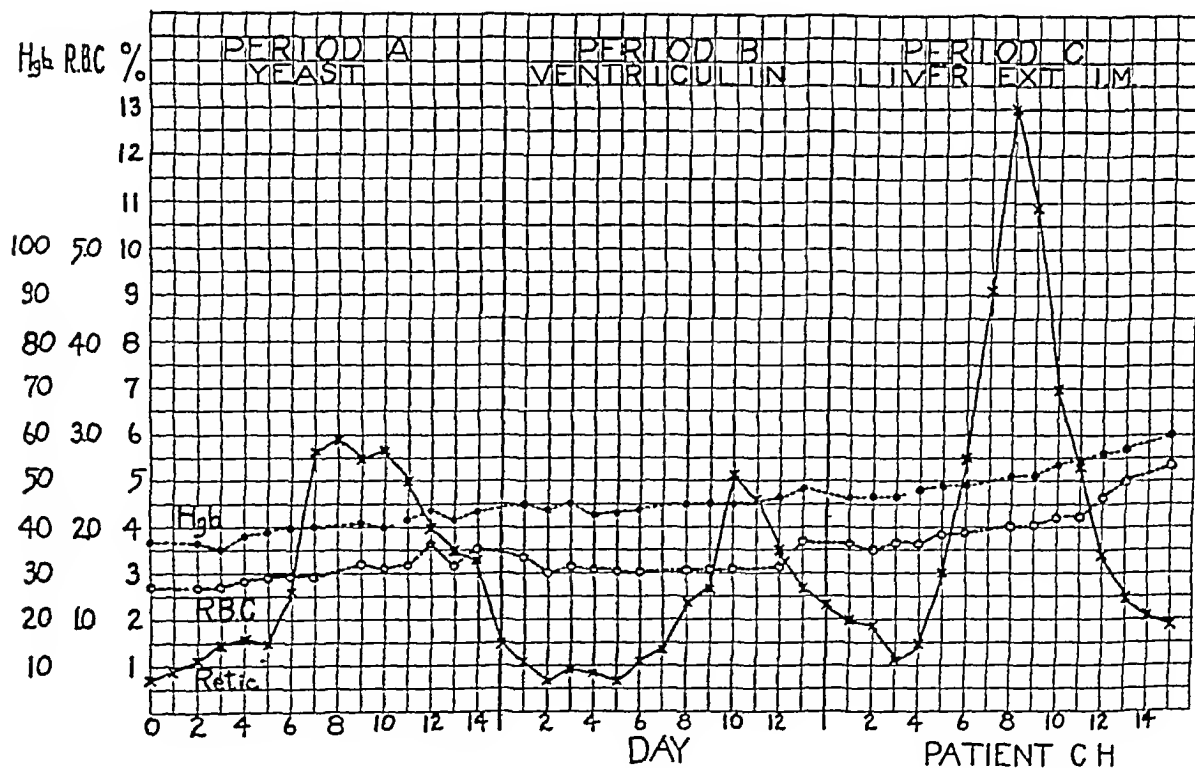


FIG. 2. PATIENT C H. COMPARATIVE HEMATOPOIETIC RESPONSE TO YEAST, VENTRICULIN AND PARENTERAL LIVER EXTRACT

red blood corpuscles and hemoglobin. The fact that small doses of parenteral liver produced a considerably greater response than a theoretically minimal dose of ventriculin calculated to evoke a maximal reticulocyte response, argues that the patient was unable to respond fully to oral medication. This does not, however, invalidate the fact that a second response to the ventriculin followed a preliminary response to yeast, but it does indicate that the ventriculin was more potent than the yeast in the dosages employed. Parenteral liver therapy in the dosage employed was considerably more effective than either the yeast or the ventriculin.

DISCUSSION

Previous work (5, 6) has demonstrated that the intrinsic factor deficiency in pernicious anemia is quantitative rather than qualitative and that the gastric juice of patients with pernicious anemia even in relapse contains intrinsic factor, but in such small amounts that it will not prevent anemia in the patient. Thus if an excess of extrinsic factor is supplied to such patients it is conceivable that enough hematopoietic substance will be formed *in vivo* to prevent anemia or at least, reduce the requirement for preformed hematopoietic substance. The response to yeast obtained in our cases is considered to be the result of supplying an excess of extrinsic factor in the presence of greatly diminished but not completely absent intrinsic factor. It is unnecessary to assume that yeast contains any hematopoietic substance specific for pernicious anemia. It supplies an abundance of extrinsic factor and certain patients with pernicious anemia may be thrown into remission with out specific therapy if an excess of such extrinsic factor is supplied. In any event, the yeast, in

the dosage employed, was not as effective as small daily oral doses of ventriculin or intramuscular liver extract.

SUMMARY

1 Two patients with pernicious anemia in relapse are reported. They obtained a response with ordinary dried brewer's yeast, but this response was neither as great as that evoked with a minimal amount of ventriculin calculated to give a maximal reticulocytosis nor with daily intramuscular injections of unconcentrated liver extract.

2 The response to yeast is due to the fact that pernicious anemia is a disease of quantitative rather than qualitative deficiency. Administration of large amounts of extrinsic factor with greatly diminished but not completely absent intrinsic factor will produce a response in certain patients with pernicious anemia.

BIBLIOGRAPHY

- 1 Wintrobe, M. M., The successful treatment of pernicious anemia by means of non autolyzed yeast. *J. Clin. Invest.* 1938, 17, 501.
- 2 Ungley C. C., The effect of yeast and wheat embryo in anemias. I. Marmite, yeastamin, and bemax in megalocytic and nutritional hypochromic anemias. *Quart. J. Med.* 1933, 2, 381.
- 3 Ungley C. C., and James, G. V., The effect of yeast and wheat embryo in anemias. II. The nature of the haemopoietic factor in yeast effective in pernicious anemia. *Quart. J. Med.* 1934, 3, 523.
- 4 Minot, G. R., and Castle, W. B., The interpretation of reticulocyte reactions. *Lancet*, 1935, 2, 320.
- 5 Goldhamer S. M., The presence of the intrinsic factor of Castle in the gastric juice of patients with pernicious anemia. *Am. J. M. Sc.* 1936, 191, 405.
- 6 Goldhamer S. M., The gastric juice in patients with pernicious anemia in induced remission. *Am. J. M. Sc.* 1937, 193, 23.

SEROLOGICAL DIFFERENTIATION OF OBSTRUCTIVE FROM HEPATOGENOUS JAUNDICE BY FLOCCULATION OF CEPHALIN-CHOLESTEROL EMULSIONS

By FRANKLIN M HANGER

(From the Department of Medicine College of Physicians and Surgeons Columbia University and the Presbyterian Hospital New York City)

(Received for publication January 4 1939)

The clinical differentiation of obstructive jaundice from that due to derangements of the liver is frequently impossible despite careful history taking and physical examination and the employment of many types of diagnostic procedures. Patients with grave hepatitis are often subjected to the added hazard of an anesthetic and laparotomy to rule out possible biliary tract obstruction, while conversely, exploration may be delayed perilously long in cases of obstruction in the hope that the jaundice will subside spontaneously.

In a previous preliminary report (1) the author has described a simple test by which disturbances in the hepatic parenchyma may be recognized by noting the capacity of the serum in these cases to flocculate a colloidal suspension of a cephalin-cholesterol complex. This test can be performed in any adequately equipped routine medical laboratory since the reagents are few and relatively stable and no costly apparatus is required.

TECHNIQUE FOR FLOCCULATION TEST

A stock solution is prepared by dissolving 100 mgm. of sheep brain cephalin¹ and 300 mgm. of cholesterol in

¹ The cephalin employed in the flocculation tests was prepared and kindly furnished by Dr Erwin Chargaff of the Department of Biological Chemistry College of Physicians and Surgeons, Columbia University. The process for preparation was as follows:

Sheep brains were dehydrated by 3 extractions with acetone and the dry tissue powder was 3 times extracted with ether (free of peroxides). The ether extracts were concentrated *in vacuo* and the crude cephalin was precipitated by the addition of 4 volumes of absolute alcohol. The resulting precipitate was dissolved in the minimum amount of ether the accompanying cerebroside impurities were precipitated by chilling and removed by centrifugation. The supernatant ether solution was again precipitated with 4 volumes of absolute alcohol, chilled, and the precipitate filtered, washed with alcohol and acetone, and desiccated. The cephalin preparation is a brown powdery material containing traces of other lipids. These, however, do not feature in the reaction.

8 cc. of ether (Squibb's anesthesia). This solution can be kept many months without deterioration in a well stoppered container. An emulsion of a cephalin-cholesterol complex may be prepared by adding (slowly and with stirring) 1 cc. of the stock ether solution to 35 cc. of freshly distilled water warmed to 65 to 70 °C. and then heating slowly to boiling. The mixture is allowed to simmer until the final volume is reduced to 30 cc. During the heating all coarse granular clumps are dispersed to a stable, milky translucent emulsion and all traces of ether are driven off. After cooling to room temperature the preparation is ready for testing which consists of adding 1 cc. of the emulsion to a test tube (preferably a centrifuge tube) containing 0.2 cc. of the patient's serum diluted with 4 cc. of normal (0.85 per cent) saline. After thorough shaking and stoppering with cotton the tube is allowed to stand undisturbed at room temperature and notation is made at the end of twenty four and forty eight hours as to the amount of flocculation and precipitation that has taken place. With normal human sera the emulsion remains as a stable homogeneous suspension but with sera from patients with diffuse hepatitis the lipid material tends to flocculate and precipitate to the bottom of the tube. A ++++ reaction indicates a complete precipitation leaving the supernatant liquid water clear. Gradations of the reaction between negative and ++++ are designated in terms of +, ++, and +++. No test should be regarded as negative until forty eight hours have elapsed without flocculation.

Few precautions are necessary. The serum should preferably be fresh or preserved at ice box temperature. Plasma also may be used but the presence of various anticoagulants creates uncontrollable uncertainties. The lipid emulsion, if properly prepared, remains stable for many days but comparable results can be expected only if it is prepared freshly on the day it is to be used. It is important to measure accurately the various ingredients and to employ only carefully washed glassware. Traces of heavy metals or strong acids may give rise to erroneous positive flocculation.

Diagnostic significance

Over nine hundred sera from normal individuals or from patients with no demonstrable hepatic disorders have been examined in the manner described and with the exception of one

TABLE I
Summary of flocculation reaction in 25 cases of jaundice due to obstruction of the extrahepatic biliary tract

Case	Initials	Sex	Age	Clinical diagnosis	Ceph- alin floccu- lation	Basis for diagnosis	Approx- imate duration of jaundice before blood analysis	Bilirubin mgm per 100 cc	Serum phos- phatase Bodansky units per 100 cc	Total protein grams per 100 cc	Albumin grams per 100 cc	Globulin grams per 100 cc	Comments
1	T C	F	36	Chronic cholangitis	0	Liver biopsy	8 months	2.4	9.7	7.1	4.3	2.8	Patient died 7 days postoperatively
2	P R	F	75	Acute cholecystitis, perithreatitis	+	Operation	10 days	13.5	not done		not done		
3	I M	F	29	Chronic cholangitis	0	Operation	3 months	3.2	36.4	6.4	4.2	2.2	
4	H L	M	55	Stones in common bile duct	0	Operation	1 month	3.4	27.6		not done		Non filling gallbladder by x ray
5	S K	M	28	Acute cholecystitis, cholelithiasis	0	Operation	2 days	7.0	7.6	7.3	4.4	2.9	
6	S K	F	50	Acute cholecystitis, cholelithiasis	0	Operation	8 days	6.0	11.9	6.7	4.3	2.4	
7	A M	F	76	Chronic cholangitis	0	Clinical	2 weeks	2.5	17.1		not done		Non filling gallbladder by x ray
8	M A	F	65	Stone in common bile duct	0	Operation	2 weeks	10.0	20.9	6.8	3.8	3.1	
9	C G	F	54	Acute cholecystitis, cholelithiasis	+	Operation	8 weeks	12.1	17.5	6.7	4.1	2.6	
10	S H	M	61	Chronic cholecystitis, cholelithiasis	0	Clinical	1 year	8	4.8				Achole stools Typical history
11	M C	F	43	Acute cholecystitis, stone in common bile duct	0	Operation	8 days	3.3			not done		
12	H L	M	72	Stones in common bile duct	0	Clinical	2 days	5.3	10.6	6.9	not done		
13	M C	M	60	Chronic cholecystitis, stones in com- mon bile duct	0	Operation	4 months	2.5	17.2		not done		Slight biliary cirrhosis Died 6 weeks after onset of jaundice
14	M P	F	72	Carcinoma, primary site not deter- mined	0	Clinical	4 months	3.4	27.6	6.4	4.2	2.2	
15	N B	M	60	Carcinoma of stomach, invasion of common duct by tumor mass	0	Autopsy	3½ weeks	10.8	14.5	5.35	3.54	1.81	
16	W G	M	49	Carcinoma of head of pancreas	0	Operation	2 weeks	7.4	16.9	6.1	4.2	1.9	Patient inoperable Died 2 months after onset of jaundice
17	L K	M	65	Carcinoma of head of pancreas	±	Operation	3 weeks	16.6	11.8	6.7	2.9	3.8	
18	S S	F	50	Carcinoma of head of pancreas	0	Operation	2 weeks	20.8	17.8	7.6	3.6	4.0	
19	I H	F	41	Carcinomatous of bile ducts	0	Operation	4 months	4.7	25.8	7.23	4.32	2.92	Postoperative stenosis of bile duct for 4 years Frequent attacks of fever, chills, leukocytosis and jaundice Observa- tion made during attack
20	I H	F	60	Carcinomatous of hepatic ducts	0	Autopsy	4 months	20.6	8.7	6.0	3.2	2.8	
21	M A	M	61	Carcinoma of esophagus, common duct occluded by tumor mass	0	Autopsy	1 month	18.8	41.0	4.8	2.5	2.3	
22	J S	M	73	Carcinoma of head of pancreas	0	Clinical	3½ weeks	17.8	29.1	6.3	3.57	2.46	Postoperative stenosis of bile duct for 4 years Frequent attacks of fever, chills, leukocytosis and jaundice Observa- tion made during attack
23	T G	M	45	Postoperative occlusion of common bile duct	0	Operation	1 week	6.8	4.76	6.3	3.3	3.0	
24	R M	F	34	Postoperative stenosis of common bile duct, relapsing cholangitis	±	Operation	2 days	10.0	4.9	7.4	4.1	3.3	
25	R R	F	27	Postoperative obstruction of common bile duct, cholangitis	0	Operation	1 month	10.1	7.8	4.9	3.1	1.8	

healthy medical student, no significant flocculation reactions have been observed.

In a like manner the sera from twenty five cases of obstructive jaundice have been tested and these also, irrespective of the cause of the obstruction, have failed to produce significant flocculation (Table I). In several instances a \pm or $+$ reaction has been noted in the forty eight hour reading. This finding presumably indicates mild hepatitis associated with the obstructive process. Even in cases of long standing biliary obstruction with secondary fibrosis in the liver the test usually remains negative.

In contrast to the findings in obstructive jaundice the serum from thirty three of thirty-eight cases of jaundice due to hepatitis catarrhal jaundice, and cirrhosis have produced a prompt, strong, flocculation reaction (Table II). The diagnosis has been established in these cases by histological examination of the liver tissue obtained at operation or autopsy, or by a clinical course so typical of catarrhal jaundice that there is little question as to the nature of the disorder.

A number of these cases have been tested repeatedly during the course of their disease and a close correlation always has been demonstrable between the clinical severity and the degree of flocculation. The following brief case report illustrates this point.

Case 4 (Table II) Hospital Number 410158 Male, age 28 years occupation—clerk.

Diagnosis Acute hepatitis—unknown etiology

Patient was admitted to the Clinic, January 5 1938, complaining of jaundice for 1 week. He had always been healthy had had no indigestion, no exposure to industrial poisons no previous medications used alcohol infrequently. There was no history of preceding infection.

Present illness began ten days previously with malaise, anorexia, and nausea. Three days later patient noted light stools and dark urine. Increasing jaundice had been present for the past week. There was no pain over the liver. Slight itching of the skin was present.

Physical examination Patient was intensely jaundiced, well nourished, and well developed. Sensorium was clear. Liver edge was 2 cm. below the costal margin, and not tender. Spleen was not palpable. There was no ascites.

Clinical course Patient was maintained on a high carbohydrate régime, and jaundice gradually subsided during the ensuing month. There was no fever no anemia, and no leukocytosis. Wassermann was negative sedimentation rate 6 mm. in 1 hour. Stools were light but positive for urobilin by the mercuric chloride test. Urine showed decreasing amounts of bile during the

month of jaundice. Patient's health has been excellent since illness.

Chemical studies of the blood in correlation with the flocculation test are shown in Table III. It will be noted in this case that the flocculation test agrees well with the clinical course while at the same time the total protein and the albumin-globulin ratio show no significant alterations.

The cephalin-cholesterol flocculation test is also of value in those instances where the clinical data are confusing or actually misleading.

Case 20 (Table II) Hospital Number 558088 Male age 63 years occupation—cabinet maker

Complaint Deepening jaundice for one month. General health was always excellent until six months previously when he first noticed a sensation of fullness across the epigastrium after meals. There was no actual pain. The attacks usually lasted about one hour and subsided spontaneously. One month before entering the clinic he noticed jaundice, light colored stools, and dark urine. His appetite became poor bowels remained regular. He lost about ten pounds in weight. There was no previous exposure to drugs or chemicals.

Physical examination The patient was an obese elderly Dutchman showing recent loss of weight. He was moderately jaundiced but did not complain of itching. A firm mass was palpable in the upper right quadrant which could be taken for either liver or an enlarged gallbladder. The spleen was not felt. There was no demonstrable ascites.

Laboratory findings Hemoglobin 15.4 grams per cent erythrocytes 5,050,000 leukocytes 7,000 Wassermann negative stools acholic urine contained bile. Serum bilirubin was 14.1 mgm. per cent, serum phosphatase 4.1 Bodansky units serum protein 6.6 per cent serum albumin 3.2 per cent serum globulin 3.4 per cent and blood amylase 19.8 units.

The presumptive diagnosis was obstructive jaundice. Support for this opinion was strengthened by the x ray finding of distortion of the second portion of the duodenum compatible with an infiltrative lesion in the region of the pancreas. The cephalin flocculation test was $++++$ a finding to be expected with intrahepatic disease rather than with biliary obstruction.

The patient was explored surgically for diagnosis. The liver was found enlarged and firm. The gallbladder was not abnormal. There was an induration in the region of the head of the pancreas (probably inflammatory in nature) which was not obstructing the common bile duct.

A section of the liver taken at operation revealed hepatitis and cirrhosis of the portal type. The lobules were irregular in size and shape. The liver cells varied in appearance. A number were vacuolated and some contained bile pigment. An appreciable number of the cells contained two or more nuclei. The connective tissue septa were unusually thick and prominent. There was

TABLE II
Summary of flocculation reaction in 38 cases of jaundice without demonstrable obstruction of the extrahepatic biliary tract

Case	Initials	Sex	Age	Clinical diagnosis	Cephalin flocculation	Basis for diagnosis	Approximate duration of jaundice		Bilirubin mgm per 100 cc	Serum phos phatase Bodansky units per 100 cc	Total protein grams per 100 cc	Albumin grams per 100 cc	Globulin grams per 100 cc	Comments
							Before blood analysis	Total duration						
1	R R	M	22	Catarthal jaundice	++++	Clinical	1 day	14 days	5.4	7.3	not done	3.94	2.99	Complete recovery
2	F B	F	37	Catarthal jaundice	++++	Clinical	10 days	20 days	6.9	3.1	not done	3.85	2.12	With clinical improvement, flocculation reaction diminished
3	L C	F	24	Catarthal jaundice	++++	Clinical	7 days	21 days	4.2	4.8	6.93	4.9	2.6	Protocol given in text
4	G M	M	28	Catarthal jaundice	++++	Clinical	4 days	42 days	7.5	7.6	6.27	3.85	2.12	Flocculation positive after clearing of jaundice, gradually de-
5	M L	F	29	Catarthal jaundice	++++	Clinical	5 days	11 days	9.6	6.5	8.5	4.9	3.6	creased with clinical improve-
6	A P	M	34	Catarthal jaundice	++++	Clinical	7 days	15 days	9.4	5.4	7.1	4.5	2.6	ment
7	F B	M	39	Catarthal jaundice	++	Clinical	14 days	20 days	4.4	5.6	7.6	4.9	2.7	Jaundice clearing when first flocculation test was made
8	H M	F	67	Catarthal jaundice, hepatitis	++++	Clinical	21 days	4 months	12.5	7.6	6.4	3.0	3.4	After 4 months jaundice clearing
9	C A	F	63	Fatal hepatitis	++++	Clinical	14 days	5 months	7.5	7.9	6.4	3.3	3.1	ing flocculation test +
10	O G	F	39	Hepatitis 24 days postpartum	++++	Clinical	14 days	2 months	9.0	9.6	7.55	4.14	3.41	Had a jaundice free remission but flocculation reaction remained +++
11	M P	F	41	Hepatitis, chronic cholelithiasis	++++	Operation	9 days	3 weeks	7.5	7.3	not done	not done		Gradual recovery with degree of flocculation paralleling clinical course
12	J R	M	48	Fatal hepatitis	++++	Clinical	30 days	2 months	12.3	7.1	6.6	2.8	3.8	Chronic cholecystitis and cholelithiasis found at operation
13	A S	M	32	Hepatitis	++++	Clinical	30 days	4 months	19.0	4.2	6.3	4.1	2.2	No obstruction
14	D H	F	52	Fatal hepatitis	++++	Autopsy	5 days	3 months	9.5	8.5	6.4	3.1	3.2	Died in cholemia
15	J L	M	61	Hepatitis suppurative cholangitis?	++++	Clinical	8 days	17 days	17.0	9.9	6.2	3.8	2.4	Strong flocculation persisted throughout illness
16	S. T.	F	21	Hepatitis	++++	Operation	5 months	9 months	18.0	12.5	9.5	1.7	7.8	Apparent recovery
17	M S	F	31	Hepatitis with cirrhosis	++++	Clinical	2 weeks	3 weeks	7.5	4.1	6.3	3.0	3.3	Protocol given in text
18	T. T.	M	47	Hepatitis with cirrhosis	++++	Clinical	3 weeks	3 months	7.2	12.0	6.6	2.6	4.0	Had a jaundice free remission but flocculation reaction remained +++
19	C A	F	67	Cirrhosis	++++	Clinical	3-4 weeks	2 1/2 months	4.6	3.0	6.2	3.2	3.0	Gradual recovery with degree of flocculation paralleling clinical course
20	H T	M	63	Hepatitis with cirrhosis	++++	Biopsy of liver	4 weeks	7 weeks	5.4	4.1	6.6	3.2	3.4	Chronic cholecystitis and cholelithiasis found at operation
21	L P	F	46	Laennec's cirrhosis slight jaundice	++++	Clinical	Not noticed by patient	Intermittent for 3 years	2.0	5.4	7.07	3.82	3.25	No obstruction
22	B S	F	35	Cirrhosis of liver Banti's syndrome	++++	Operation			2.5	20.0	8.1	3.6	4.5	Died in cholemia

TABLE II—Continued

Case	Initials	Sex	Age	Clinical diagnosis	Cephalin flocculation	Basis for diagnosis	Approximate duration of jaundice		Bilirubin mg. 100 cc.	Serum phosphatase Reactivity per 100 cc.	Total protein grams 100 cc.	Albumin grams 100 cc.	Globulin grams 100 cc.	Comments
							Before blood analysis	Total duration						
23	M. C.	F.	43	Leucosis + cirrhosis; slight jaundice	+++++	Autopsy	Intermittent over 1 year		2.0	3.6	8.5	2.1	6.41	Died 14 years after first symptoms of ascites
24	M. B.	F.	52	Cirrhosis of liver; Band's syndrome	+++++	Clinical	Intermittent over 1 year		2.0	9.8	6.70	2.0	4.70	Ascites, large liver and spleen; jaundice followed by ascites; retention after 4 hours
25	C. S.	M.	25	Leucosis + cirrhosis; cholelithiasis	++	Operation	2 days	4 days	2.0	13.2	6.5	3.7	2.8	Acute cholelithiasis superimposed upon cirrhosis. Died 2 days postoperatively
26	M. F.	F.	32	Leucosis + cirrhosis	+++++	Clinical	Intermittent over 1 year		2.5	4.7	6.8	3.06	3.75	Strong flocculation and jaundice continued throughout illness
27	J. V.	F.	57	Hepatitis and cirrhosis	+++++	Clinical	3 months	6 months	6.5	8.5	6.8	2.1	4.7	Phosphatase negative 1 week after onset of jaundice
28	P. G.	M.	38	Acute hepatitis (alcoholic?); pancreatitis	++	Clinical	2 days	1 week	1.5	8.6	5.26	2.52	2.44	Exceptional case of hepatitis with jaundice showing negative flocculation. Protocol
29	T. S.	M.	42	Hepatitis due to alcohol?	0	Clinical	2 weeks	7 weeks	21.0	5.1	5.1	3.5	1.6	Exceptional case of hepatitis with jaundice showing negative flocculation. Protocol
30	J. L.	M.	36	Alcoholic cirrhosis; ascites also	+	Clinical	Not noticed by patient		1.5	3.5	7.2	3.3	3.9	Flocculation test done one week after acute alcoholism
31	S. G.	F.	57	Acute phosphorus poisoning	+++++	Autopsy	1 day	2 days	2.7	8.3	4.2	2.7	1.5	Jaundice appeared 6 weeks after first arsenamine injection
32	C. S.	M.	21	Acute tuberculosis of liver	+++++	Autopsy	3 days	3 weeks	4.7	26.6	5.2	3.0	2.2	Took fatal dose of rat poison
33	V. P.	F.	35	Hepatitis following arsenamine	+++++	Clinical	3 weeks	10 weeks	13.0	6.9	6.5	3.1	3.4	Miliary tuberculosis with lesions especially numerous in the liver
34	E. P.	M.	41	Hepatitis following arsenamine	+++++	Clinical	7 days	3 weeks	13.0	4.1	6.5	3.8	2.7	Jaundice 3-4 months after first arsenamine injection
35	E. T.	F.	43	Hepatitis following arsenamine	+++++	Clinical	7 days	3 weeks	11.7	7.3	6.0	3.4	2.6	Jaundice appeared 8 months after first arsenamine injection
36	J. G.	M.	19	Hepatitis following arsenamine	+	Clinical	6 days	3 weeks	7.0	11.4	6.85	3.6	3.26	Stomach upset and jaundice few hours after third arsenamine injection
37	E. C.	F.	36	Hepatitis following arsenamine	0	Clinical	4 months	6 months	9.4	13.9	6.9	3.9	3.0	Stomach upset and jaundice in 2 weeks after third arsenamine injection
38	A. A.	M.	29	Hepatitis following arsenamine	0	Clinical	5 days	3 months	17.0	37.6	6.5	3.4	3.1	Stomach upset immediately after 26 arsenamine injections in 3 months; jaundice 4 days after Protocol given in text

TABLE III

Blood chemistry studies, Patient 410158, showing correlation with cephalin-cholesterol flocculation test

Date (1955)	January 6	January 12	January 20	January 29	February 7	February 14	April 26
Serum bilirubin, mgm per 100 cc	12.1	17.0	17.6	7.5	3.8	2.5	
Serum nonprotein nitrogen, mgm per 100 cc	26			27		21	
Serum phosphatase, Bodansky units per 100 cc (Normal 1 to 4 units)	8.8			7.6		2.9	
Serum cholesterol, mgm per 100 cc				309			
Serum sugar, mgm per 100 cc				77			
Serum total protein, grams per 100 cc	7.0			6.3			
Serum albumin, grams per 100 cc	4.2			3.9			
Serum globulin, grams per 100 cc	2.8			2.4			
Degree of icterus	Intense	Intense	Intense	Clearing	Slight	Slight	Completely absent
Flocculation test	++++	++++	Not done	++	+	0	0

marked irregular proliferation of the bile duct epithelium. About the biliary ramifications in the fibrous connective tissue were inflammatory cell foci composed predominantly of plasma cells, lymphocytes, and rare polymorphonuclear leukocytes. No bile was seen in the ducts. The patient's jaundice cleared spontaneously. He has been symptom-free for the past three months.

Prognostic significance

The flocculation test is of considerable value prognostically in cases of hepatitis. Many patients with mild attacks develop negative reactions before the icterus has completely subsided. For this reason, performing the test late in the course of the disease may yield little of differential diagnostic value. However, finding a negative flocculation at this stage is a favorable indication, since no relapses or evidence of persisting liver disease have yet been observed in a case of acute hepatitis once the reaction has returned to normal. On the other hand, the maintenance of a strongly positive flocculation reaction, irrespective of changes in the degree of jaundice, is of grave import and is usually indicative of active progressive liver degeneration.

The following abstract indicates the typical findings in a case of intractable hepatogenous jaundice.

Case 14 (Table II) Hospital Number 557929 Female, age 52 years, housewife.

Patient lived in Colombia, South America, until the age of twenty and has had no serious illnesses except chronic malaria in early adult life. General health was excellent. She has used considerable alcohol (7 oz. gin) daily for years. Her diet in the past year has been inadequate and especially low in vitamin content. She has lost 20 pounds in this interval and has noticed progressive

weakness. Three months before admission to the clinic, paresthesias, and hyperesthesias developed in the lower extremities. One month later she complained of anorexia, constipation, belching, distention, and attacks of sharp intermittent epigastric pain. Five days before admission she noticed jaundice and dark urine for the first time. The stools remained brown. There was no itching of the skin.

Physical examination. The patient was an emaciated, tired looking, middle-aged woman. She was moderately jaundiced and showed on the face and body a number of spider angiomas. The liver, which was enlarged 8 cm. below the costal margin, seemed firm and irregular. The spleen was not palpable. The knee jerks and ankle jerks were absent, and there was considerable hyperesthesia of the lower limbs.

Course. A painful friction rub soon appeared in the region of the liver and ascites requiring frequent tapings developed. She became progressively weaker and more jaundiced in spite of high carbohydrate administration and large intake of vitamins both in the food and parenterally. She died in stupor three months after the onset of jaundice.

Laboratory findings. Hemoglobin 8 grams per cent, erythrocytes 2,590,000, leukocytes 16,400, polymorphonuclears 86 per cent, erythrocyte sedimentation rate 82 mm. in 1 hour. Urine was negative except for choloria. Stools were brown and contained urobilin, no occult blood was present. X-ray examinations of the chest and gastro-intestinal tract revealed no evidence of malignancy. Wassermann was negative, gastric analysis, free HCl absent, serum bilirubin, 9.5 mgm. per cent direct, serum phosphatase 8.5 Bodansky units, blood cholesterol 208 mgm. per cent, serum protein 6.2, serum albumin 3.1 per cent, serum globulin 3.2 per cent, and euglobulin 0.6 per cent. The bromsulphalein test showed 60 per cent dye retention after 30 minutes. Takata-Ara test +++.

The cephalin flocculation test was performed one week after the onset of jaundice and again a short time before death. In both instances the reaction was strongly posi-

tive. In the meantime total serum protein dropped to 5.2 per cent, serum albumin to 2.3 per cent, and serum globulin to 2.9 per cent.

Histologically, the liver lobulation was completely distorted. There was central and periportal fibrosis with marked polymorphonuclear leukocytic infiltration in the periportal connective tissue. The most prominent feature was the jaundice and necrosis of the more central liver cells. Many had hyaline droplets and occasionally others showed leukocytes within the boundaries of the disintegrating cells. There was proliferation of bile ducts in the portal areas, many of which were also infiltrated by polymorphonuclear leukocytes.

The flocculation test in hepatitis due to known toxic agents

Patients with jaundice following administration of arsphenamine may be indistinguishable by flocculation reactions from the idiopathic types of hepatitis already described. On the other hand of the four instances in our records of hepatitis with icterus failing to give a positive reaction, three are attributable to the recent use of this drug.

The following abstract deals with this type of case.

Case 38 (Table II) Hospital Number 551044 Male age 29 years occupation—cashier

Patient's general health was excellent. Initial injection of arsphenamine had been two months previously for an asymptomatic positive Wassermann reaction. There had been no discomfort after the first injection but about four hours after the second treatment, one month later he developed a chill, vomited and became prostrated. He continued to run fever and developed puffy eyes, anorexia, and pain across the upper abdomen. Jaundice was noticed five days after the arsphenamine administration accompanied by itching, dark urine, and light stools.

Physical examination. Patient was deeply jaundiced. There was distressing pruritis of the skin. Liver and spleen were not palpable.

Course. Patient's jaundice subsided slowly over a period of three months. During this period he ran an irregular fever of about 100. He has subsequently made a satisfactory recovery.

Laboratory findings. Hemoglobin 12 grams per cent erythrocytes 4,250,000 leukocytes 8,400 polymorphonuclears 58 per cent eosinophiles 8 per cent. Stools were acholic at onset, but contained bile later. Erythrocyte sedimentation rate was 113 mm. in one hour. The urine showed a heavy trace of bilirubin. X-ray examinations subsequently have revealed a normal gallbladder. Serum phosphatase was 17.6 Bodansky units serum bilirubin 17.0 mgm. per cent serum cholesterol 580 serum protein 6.5 per cent serum albumin 3.4 per cent serum

globulin 3.1 per cent. Chemical studies made weekly during the course of his illness showed a gradual return to more normal figures.

The flocculation test on this patient was repeatedly negative.

In contrast to the group of post arsphenamine cases showing strong flocculation reactions and resembling idiopathic hepatitis the negatively reacting group is characterized by prompt onset of disability and jaundice following arsphenamine injection, intense itching of the skin, little disturbance of the albumin globulin ratio and elevated serum phosphatase. Flood, Gutman, and Gutman (2) have already noted the elevation of serum phosphatase in about half of their cases of post arsenical hepatitis and have pointed out that the high values encountered would accord better with an obstructive process. The absence of flocculation also supports an assumption that the mechanism of jaundice production in certain of the post arsphenamine cases is different from that operating in idiopathic hepatitis. It is of practical importance that a negative flocculation test may lead to the erroneous diagnosis of obstructive jaundice in patients who have recently received arsphenamine.

Instances of liver damage due to other recognized toxic agents have been too few to warrant any comparison with the post-arsenical group. One case of fatal phosphorus poisoning (Case 557927) showed a ++++ reaction. On the other hand, a single unexplained case of hepatitis with jaundice, which followed soon after acute alcoholism, and gave a negative flocculation test, has been encountered.

Case 29 (Table II) Hospital Number 557966. Male age 42 years occupation—fireman.

Two days following heavy overindulgence in beer and whiskey the patient noticed dark urine and light stools followed by jaundice. He vomited once. There was moderate itching of the skin, but no abnormal bleeding. General health always had been excellent. Patient has used alcohol regularly for years with occasional episodes of excessive drinking. He had had no previous medications nor exposure to injurious chemicals. Diet had been adequate. He entered the clinic after increasing jaundice for two weeks.

Physical examination. Patient was well nourished, afebrile, and intensely jaundiced. The liver extended to the level of the umbilicus and was not tender. Tip of the spleen was palpable. There was questionable ascites and slight pitting edema of the ankles.

Course Jaundice gradually cleared in seven weeks and liver decreased considerably in size. Patient has been well and active since recovery.

Laboratory findings Hemoglobin 14 grams per cent, erythrocytes 4,400,000, leukocytes 6,600, polymorphonuclears 74 per cent, erythrocyte sedimentation rate 3 mm in one hour. Urine was negative except for bile +++++. Stools contained urobilin. X-ray of the gastro-intestinal tract revealed no abnormalities. Kline test was negative. Serum bilirubin was 210 mgm. per cent, serum phosphatase 51 Bodansky units, serum cholesterol 121 mgm per cent, serum protein 51 per cent, serum albumin 35 per cent, serum globulin 16 per cent. Cephalin flocculation was repeatedly negative.

A negative reaction would have been more significant had it been obtained earlier in the course of his disease, since a number of non-jaundiced, acute alcoholics with temporary enlargement of the liver have shown a positive flocculation reaction during a brief period only. It is suggestive, however, that the patient was suffering from a toxic hepatitis due to alcohol and that the mechanism of jaundice production may differ with the various types of liver injury.

The flocculation test in jaundice due to miscellaneous causes

Cases of jaundice due to cholangitis, liver abscess, and new growth have been too few in our series to establish an evaluation of the flocculation test in these conditions. Single or circumscribed suppurative lesions in the liver are associated generally with a negative reaction while cases in which the process is multiple or widely disseminated give a positive reaction. Carcinomatosis of the liver, both primary and metastatic, usually is accompanied by a negative reaction. Several cases, however, with metastases in the liver from primary tumors of the colon, esophagus, *etc.*, have shown a positive reaction.

All four cases of hemolytic jaundice studied have failed to show flocculation. On the other hand, jaundice appearing during acute or chronic infections such as pneumonia or septicemia is usually accompanied by a positive test. This finding tends to indicate that icterus occurring during infection is secondary to changes in the liver rather than to excessive blood destruction as is sometimes assumed.

Comparison of the flocculation reaction with liver function tests

With the exception of a few post-arsphenamine cases, the cephalin-cholesterol flocculation test has proved to be a more sensitive and more accurate

index of *active* disturbances of liver parenchyma than any of the so-called liver function tests. Occasionally, hepatic insufficiency may be demonstrable by poor bromsulphalein excretion or other methods when the flocculation test is consistently negative. This has been observed chiefly among patients with extensive neoplastic involvement or with chronic cirrhosis in whom the process is stationary so far as can be determined by long periods of observation. It is probable that the replacement of active liver cells by new growth or scar tissue in these instances, rather than inflammatory or degenerative processes, accounts for the impairment of hepatic function. Like the finding of albuminuria, which is often a better early indication of active kidney disease than renal function tests, so, a positive flocculation may disclose certain types of liver derangements when the usual liver function tests are equivocal. Comparisons with other procedures have not been made in all cases, but no strict correlation of the degree of flocculation has been established with the bromsulphalein excretion, hippuric acid formation, or levulose tolerance test. Neither does it necessarily parallel the Takata-Ara reaction, the formol gel test, the serum bilirubin or serum cholesterol content, or quantitative changes in any of the recognized protein constituents. It may be positive or negative irrespective of the Wassermann reaction or erythrocyte sedimentation rate.

Mechanism of the flocculation reaction

The mechanism of the flocculation test is still under investigation and will be reported elsewhere. The factors upon which the phenomenon depends are probably comparable to those involved in the flocculation tests for syphilis as described by Eagle (3). In brief, there is evidence supporting the assumption that a strongly flocculating serum contains a nitrogen bearing constituent in the globulin fraction, which, during the reaction, becomes attached to the surface of the cephalin-cholesterol particles. The film of adsorbed protein probably brings about changes in surface potential and increase of cohesive forces between the colloidal elements. The flocculation reaction depends upon the specific properties of certain cephalins. It is not obtained with cholesterol or cephalin alone or with emulsions composed of cholesterol and other

lipids such as egg lecithin. The role of cholesterol in the reaction is probably that of orientating and furnishing a vehicle for the cephalin component. The nature of the flocculating substance and the reason for its presence during the course of certain hepatic disturbances is still speculative.

CONCLUSIONS

1 Emulsions prepared from mixtures of sheep brain cephalin and cholesterol *are not* flocculated usually by normal human serum or by serum from patients with obstructive jaundice.

2 Cephalin-cholesterol emulsions *are* flocculated by sera from patients with active disturbances of liver parenchyma.

3 Jaundice due to biliary obstruction may be distinguished usually from hepatogenous jaundice by the cephalin-cholesterol flocculation test.

4 The degree of flocculation parallels the severity of active liver disease and may therefore be employed prognostically in estimating the degree and persistence of the active process

5 Certain patients with jaundice appearing immediately following salvarsan injections may give a negative flocculation reaction and thus simulate obstructive jaundice.

6 The flocculation test may be regarded as an index of disturbance of liver parenchyma and does not parallel hepatic function tests

7 The mechanism of the flocculation test probably depends upon the capacity of an altered globulin constituent of the serum to become affixed to the colloidal elements of the emulsion.

BIBLIOGRAPHY

- 1 Hanger, Franklin M. The flocculation of cephalin-cholesterol emulsions by pathological sera. *Tr. A. Am. Physicians* 1938 53 148.
- 2 Flood, Charles A., Gutman, Ethel Benedict, and Gutman, Alexander B. Phosphatase activity inorganic phosphorous and calcium of serum in disease of liver and biliary tract. *Arch. Int. Med.*, 1937 59 981
- 3 Eagle, Harry. *Laboratory Diagnosis of Syphilis*. Mosby St. Louis, 1937

THE EFFECT OF SLEEP ON SKIN TEMPERATURE REACTIONS IN A CASE OF ACROCYANOSIS

BY RICHARD DAY AND WALTER O. KLINGMAN

(From the Normal Child Development Study of the Department of Diseases of Children and the Department of Neurology, Columbia University and the Babies Hospital, New York City)

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Acrocyanosis is a condition in which the extremities are cold and blue. It differs from Raynaud's disease in that it does not occur in attacks but is relatively permanent, and in that there is no sharp line of demarcation separating the normal skin from the abnormal areas. Lewis and Landis (1) believe that the fault lies in an excessive sensitivity to cold on the part of the small vessels of the extremities. This sensitivity results in spasm of the arterioles and a secondary dilation of the venous capillaries. Villaret and co-workers (2) and Kreindler and Elias (3) believe that the cause is hypertonicity of the sympathetic nervous system. Elliott Evans and Stone (4) emphasize chiefly a local fault of the small vessels but they recognize that centrally arising impulses may be contributory. They feel that there is merely dilation and atonicity of the venules and venous capillaries rather than spasm of the arterioles. Pearce (5) believes that in Raynaud's disease sensitivity of the vessels causes an exaggerated vascular response to normal influences, whether local or general.

It is the purpose of this paper to describe a study of the spontaneous warming of the hands and feet in sleep in a case of acrocyanosis. The facts collected lend support to the theory that the condition in this case may be the result of vasoconstrictive influences arising from central portions of the sympathetic nervous system and not directly related to the temperature of the environment.

CASE HISTORY

The patient, M. L. (Babies Hospital Number 546 672) was a 6½-year-old girl of Russian Jewish parentage. A sibling who had died and a cousin who was otherwise well had cold hands and feet. Our patient had suffered from a predominating coldness and blueness of her hands and feet since the age of six months with partial relief in the summer but with complete relief at all seasons during sleep. She also had periodic episodes of unex-

plained vomiting lasting two or three days which were the occasions for her two admissions to the hospital. Abnormal physical findings at 6½ years of age were confined to the skin. The hands and feet felt very cold. The hands were cyanotic, being about VIII to X on Lewis scale (6). They were slightly damper than normal. The feet were less affected than the hands. The abnormal skin of the hands and feet merged gradually with that of the normal legs and forearms. The remainder of the skin was normal except for irregular red areas which appeared when the child had cried hard for a few minutes. They would appear all over the body and resembled, except in distribution, an ordinary blush. The blood pressure was 105/70 pulse 90 and respirations 20 per minute. The IQ was 70 on the Stanford Binet scale and there was definite emotional instability.

Blood counts, urine analyses, Mantoux test, and blood Kahn test were normal. X rays showed no cervical ribs. During one of her vomiting episodes, x rays following a barium meal were taken and Dr. John Caffey's report states "Roentgen findings demonstrate complete pyloric obstruction for 3 hours and gastric retention of considerable amount at 6½ hours." That this obstruction resulted from spasm is indicated by the complete disappearance of this block when roentgen examination was repeated. The child had no digestive symptoms while in the hospital and further investigation was confined to a study of skin temperatures.

SPECIAL OBSERVATIONS

Measurements of the rectal and skin temperatures were made under various circumstances. For the skin temperatures a thermocouple was used which was accurate to about 0.5° C. Rectal temperature was continuously recorded by a nickel resistance thermometer accurate to 0.1° C.

The prompt rise in finger and hand temperature following the onset of normal sleep is shown in Figure 1. The cheek cooled off slightly. The rectal temperature rose slightly during the first part of the observation and then remained constant. In Figure 2 there is shown the equally prompt cooling of the fingers and hand when the patient awoke from normal sleep. The child had gone to sleep with mittens on. The right hand

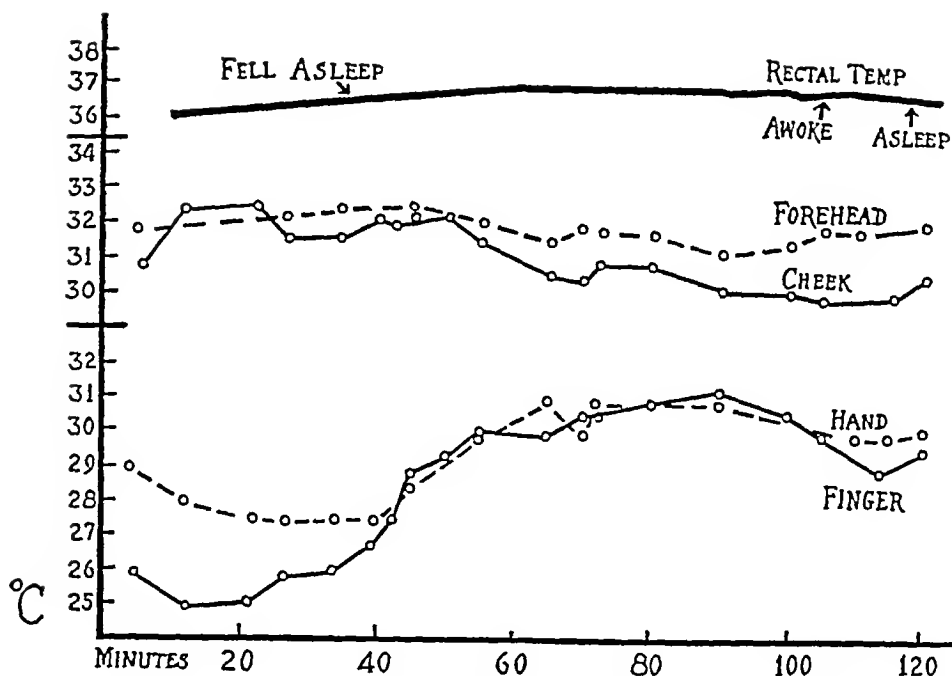


FIG 1 RAPID RISE IN THE SKIN TEMPERATURE OF THE HAND AND FINGER FOLLOWING THE ONSET OF NORMAL SLEEP

The room temperature varied between 22.8° C and 23.3° C. The child was dressed in a single cotton garment, and was covered, except for the parts measured, with a cotton sheet

mitten was removed at the beginning of the observation. The left hand mitten was kept on except momentarily for the three observations recorded on the chart, thus maintaining the same thermal environment as had been present during sleep. The elevation of skin temperature in sleep was accompanied by a change in color to a deep red which, upon awakening, was again rapidly replaced by cyanosis.

These observations suggest that in this case of acrocyanosis there is, during the waking state, a

vascular spasm which results from central impulses rather than from local conditions and that these impulses are in abeyance during sleep. To test this further, observations were made on the reactions to various thermal conditions, both local and general, when the subject was awake and when she was asleep. The results are shown in Figures 3, 4, and 5.

In Figure 3, sleep was induced by 0.240 gram of sodium phenobarbital. After the initial rise in temperature of the finger and hand, the palm of the hand was placed in contact with a cake of ice for a period of 10 minutes (*A* in Figure 3). Although the temperature of the palm of course fell (to 25° C), the dorsum of the hand showed only a slight fall, and there was no change in color. After the ice was removed (*B*) both feet were placed in tepid water (*C*), which was then raised to about 43° C. The water was then cooled, at first slowly, and then rapidly to 18.9° C (*E*). The foot bath was removed at *F*. The skin temperature of the finger, hand, and arm as well as the rectal temperature showed fluctuations during these variations in the foot bath temperature. In fact, the fall in rectal temperature induced by the

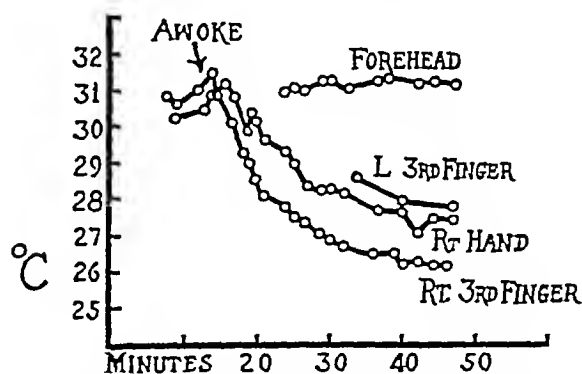


FIG 2 FALL IN SKIN TEMPERATURE OF THE HANDS AND FINGERS OCCURRING UPON AWAKENING

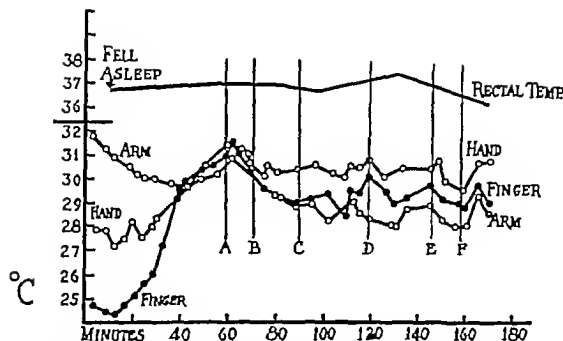


FIG. 3 EFFECT OF VARIOUS THERMAL SITUATIONS TESTED WHILE THE CHILD WAS ASLEEP UNDER THE INFLUENCE OF 0.240 GRAM OF PHENOBARBITAL

The room temperature varied between 21.6 C. and 22.7 C. and the child was dressed in a single cotton garment. For explanation of the procedures, see text.

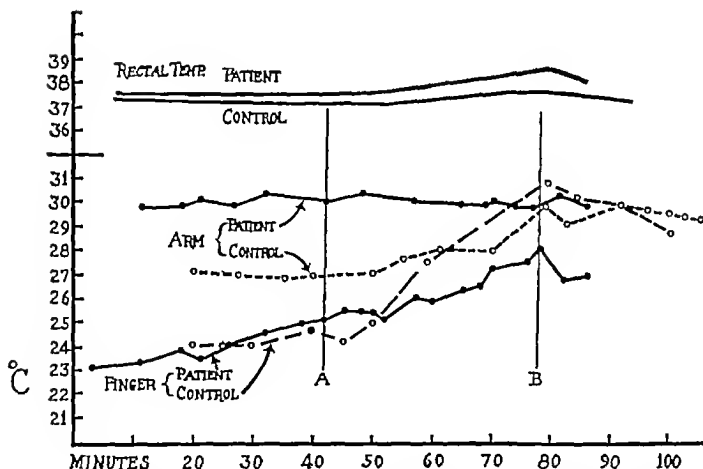


FIG. 4 PATIENT'S REACTION (WHILE AWAKE) TO A WARM FOOT BATH AS COMPARED WITH THAT OF A NORMAL CHILD

The room temperature was 23 C. for the patient, and slightly cooler (21 C.) for the control, in order to bring the skin temperature of the latter down to a level comparable with the patient's. At A the feet were put in a warm foot bath (about 42 C.) and removed at B.

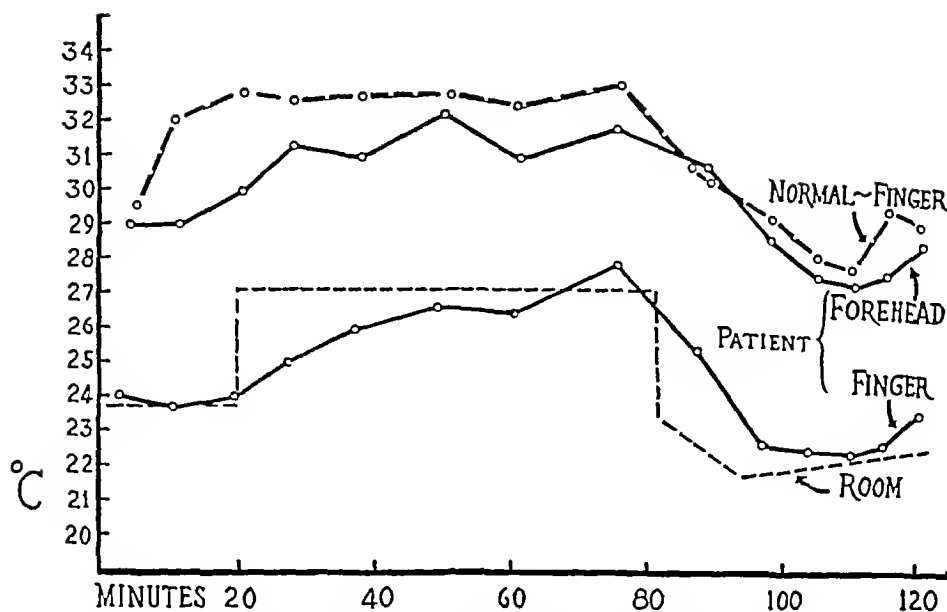


FIG 5 THE EFFECT ON SKIN TEMPERATURE OF MOVING THE PATIENT AND A NORMAL CONTROL FROM A COLD TO A WARM ROOM, AND BACK AGAIN

The room temperature is shown by the dash line. The control was an adult who was dressed in ordinary clothes, and the patient had on two simple one-piece cotton garments. The patient's finger did not deviate much from the room temperature. The amount of rise and fall in skin temperatures was about the same in the two subjects.

cool foot bath amounted to 1°C in 35 minutes. However, it should be noted that the temperature of the hand and finger varied in a parallel fashion with that of the upper arm, although the latter area was not involved in the cyanotic condition. Both constricting and dilating influences, then, are responded to by the hand in the same way as by the skin of the arm, so long as the patient is asleep. These reactions could be tested only after phenobarbital because in normal sleep the procedures awakened the patient. Even a short period of wakefulness has an effect on skin temperature, as is shown in the last portion of the graph in Figure 1.

When the patient was awake, the hand responded sluggishly to dilating influences. A standard test of autonomically mediated vasodilatation consists in noting the elevation of skin temperature of the extremity to be examined in response to heat applied to the body by, for example, a warm foot bath. Figure 4 shows the results of such a test in this patient, in comparison with a normal child. The procedure produced a delayed and incomplete vasodilatation. Figure 5 shows the response obtained by moving the pa-

tient and a control subject (a normal adult) from a cool room to a warm room and back again. It is seen that quantitatively, the rise in skin temperature was about the same in the two subjects, and about the same as the rise in the environmental temperature. Again, however, the patient showed a sluggish response. These two observations suggest that when the patient is awake, dilating influences in response to the demands of thermal regulation can occur, but they are sluggish, as though hindered by the persistent vasoconstriction that is present when the patient is awake. In the summer, it was likewise noted clinically in our patient that although the hands were warmer than in the winter, they were nevertheless at all times cooler than those of normal children.

It was noticed that during the observations made while the foot bath was being used the rectal temperature of the patient seemed more labile than that of the control subjects. To quantitate this observation, however, a larger series of normals would be required.

In order to speculate intelligently as to the nature of the constricting influences which in this patient are abolished by sleep, a better knowledge

of normal physiological changes in sleep would be required

ACTION OF DRUGS

The following drugs had no detectable influence on skin temperature or color of the hands in our patient: papaverine hydrochloride (15 mgm intravenously), padutin (0.25 cc. intramuscularly), atropine sulphate (0.3 mgm. subcutaneously), or gotamine tartrate (0.07 mgm subcutaneously). On two other occasions ergotamine tartrate seemed to be the cause of a rise of 3°C . in the skin temperature of the hand but that could not be repeated later. Syntropan (16 mgm by mouth) had no effect.

The inhalation of amyl nitrite was followed by a rise of 1°C for a few minutes.

Histamine iontophoresis was applied to one hand. A 1 per cent histamine ointment was used and a current of 1000 milliamperes seconds. The skin temperature rose from 25°C . to 29°C ., and the color of the hand became a deep red. Within an hour after the treatment, however, the temperature had fallen to 27°C ., and the cyanotic color had returned.

OTHER OBSERVATIONS

Microscopic observation of the capillaries of the nail bed showed considerable dilation on the venous side, and very little flow of blood.

Elevation of the arm caused a paling of the hand in about 15 seconds. The paling was less marked than the descriptions in Lewis and in Elliott's papers would indicate occurred in their cases. It was sufficient, however, to provide good evidence against venous block.

Placing the hand in water at 40.5°C . changed the color of the hand to a deep red. This test suggested that local heat in this case could bring about dilation and a flow of blood. The depth of color provided further evidence that the venous capillary bed was dilated. Such dilation would be in accord with the capillary microscopic findings and with the observations of both Lewis and Elliott.

When the patient was emotionally upset, as in a temper tantrum, red blotches appeared over her whole body. These were from 1°C . to 3°C higher in temperature than the surrounding skin.

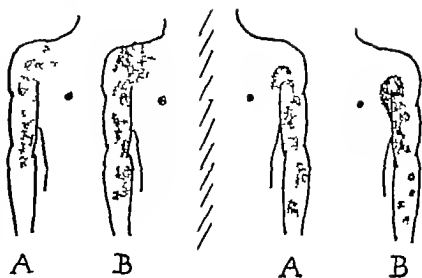


FIG. 6. DISTRIBUTION OF WARM RED AREAS ON THE ARMS

These would appear during temper tantrums and the pattern assumed by the red areas would often, but not always be repeated with each episode of crying. The diagram at A was drawn during a temper tantrum three days before the one at B.

Furthermore, as the diagram of the arms in Figure 6 shows the involved areas had a pattern which was often reproduced in a rough way on different days. On other occasions, this pattern would assume new forms. Some widespread disorder of the sympathetic vasomotor system would be required to explain this observation. The pyloric spasm noted on x ray examination might well have been sympathetic in origin also.

SUMMARY

A case of acrocyanosis in a $6\frac{1}{2}$ -year-old girl is described. In sleep her hands and feet became warm and red and under these conditions the hands responded in a parallel way with the rest of the body to warm and cold foot baths. Local cooling of the palm of the hand did not induce vasoconstriction when she was asleep. Microscopic observation of the capillaries at the base of the nails showed sluggish flow of blood and dilation of the venous side. These observations seem to support the contention of Lewis that in acrocyanosis the primary fault is vasoconstriction of the arterioles. However the predominant influence of sleep over thermal influences on the condition of this patient's hands points to abnormal vasomotor tone of central origin as the primary cause, rather than local sensitivity to cold such as was demonstrated in their cases by Lewis and by Pearse. Further evidence of

nervous system dysfunction was noted in a peculiar blotchiness of the skin during spells of anger. Spasm of the pylorus, which was demonstrated radiologically on one occasion, constituted a possible cause of her periodic episodes of vomiting, and presented further evidence of widespread sympathetic abnormality.

We wish to thank Dr. I. Shulman of the Department of Pharmacology for the capillary microscopic examination, and Dr. Robert Muller of the Physical Therapy Department of the Presbyterian Hospital for doing the histamine iontophoresis. We are also grateful to Dr. Beverly Smith for valuable criticism.

BIBLIOGRAPHY

- 1 Lewis, T., and Landis, E. M., Vascular mechanisms in acrocyanosis. *Heart*, 1930, 15, 229.
- 2 Villaret, M., Justin-Besançon, L., Cachera, R., and Boucomont, R., Étude critique sur la pathogénie des troubles circulatoires périphériques. Première Partie. Les Acrocyanoses. *Arch. d. mal. du cœur*, 1934, 27, 725.
- 3 Kreindler, A., and Elias, H., Zur Klinik und Pathogenese der juvenilen Akrocyanose. *Ztschr. f. Kinderh.*, 1930-31, 50, 608.
- 4 Elliott, A. H., Evans, R. D., and Stone, C. S., Acrocyanosis. A study of the circulatory fault. *Am. Heart J.*, 1936, 11, 431.
- 5 Pearce, Herman E., The influence of the heat regulatory mechanism on Raynaud's disease. *Am. Heart J.*, 1935, 10, 1005.
- 6 Lewis, T., Experiments relating to the peripheral mechanism involved in spasmodic arrest of circulation in fingers, variety of Raynaud's disease. *Heart*, 1929-31, 15, 7.

THE INFLUENCE OF THE FOODSTUFFS UPON THE SUSCEPTIBILITY OF THE LIVER TO INJURY BY CHLOROFORM, AND THE PROBABLE MECHANISM OF THEIR ACTION¹

By SAMUEL GOLDSCHMIDT HARRY M VARS AND ISIDOR S RAVDIN

(From the Department of Physiology and the Harrison Department of Surgical Research
School of Medicine University of Pennsylvania Philadelphia)

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In a previous publication data were presented which show that anesthesia produced by the inhalation of chloroform or divinyl ether volatilized by a stream of oxygen, results in much less damage to the liver of the dog than when air is used as the volatilizing agent (1). Evidence already presented (2) reveals in the dog no difference in the rate or the degree of discharge of hepatic glycogen or the height to which the blood sugar rises or the amount of fatty infiltration when the chloroform is volatilized with air or with oxygen provided a very marked anoxemia does not exist. The protective action of oxygen appears not to be associated with these metabolic changes.

In the course of this work our attention was directed to the relation of the dietary to the susceptibility of the animals to liver damage by chloroform and thus to the present investigation. That a diet high in carbohydrate is protective and that a diet high in fat induces maximal susceptibility of the hepatic cells when the liver is exposed to chloroform has received much confirmatory support since first reported by Opie and Alford in 1914 (3, 4, 5, 1). Diets rich in protein have, also been found to be protective against the necrotizing action of chloroform. Its value has been regarded as either inferior (3, 4) or at least equal to (5) a carbohydrate diet in ameliorating the deleterious effects of chloroform.

Fasting also intensifies to a great degree the susceptibility of the hepatic cells to injury by chloroform comparable to (4) or exceeding (5) that which results from a high fat intake.

It should be emphasized that our present studies and discussion do not include the late effects

of poisoning by chloroform. The histological evidence of hepatic injury twenty four hours following the anesthesia is for the most part, our sole criterion of the effect of the chloroform. Thus we are dealing with its primary effects; later secondary factors may enter the picture as shown by Minot and Cutler (6).

Since the liver is the organ conspicuously attacked by chloroform it is reasonable to assume that the composition of the hepatic cells determines their susceptibility to injury. Moise and Smith (5a) emphasized the desirability, in experiments of this type of establishing a nutritive equilibrium in the animal on the ration in question before producing the tissue injury. In the present experiments not only have the diets been adequate and feeding prolonged but also the hepatic glycogen and lipid concentrations have been determined analytically on control animals before, and on the experimental animals after, anesthesia with chloroform. Previous investigators have made isolated determinations of glycogen, and others have estimated the amount of fat and glycogen in the liver by histological staining. The latter method is only qualitative, and may be entirely misleading as comparisons with chemical analysis show (ourselves unpublished and others (7c, 8a)).

The white rat was chosen as the experimental animal because of the ease of attainment of adequate maintenance and growth on diets predominating in one or the other of the foodstuffs and also because of the high degree of uniformity attainable in the composition of the liver on a given ration.

A prolonged dietary régime, with chemical analyses of the resultant composition of the liver has yielded information which when correlated with the histological evidence reveals the influence of each of the foodstuffs in modifying the hepatic injury by chloroform. F

¹ Preliminary reports of part of the data in this paper were made before the Physiological Society of Philadelphia (Am. J. M. Sc. 1937 193 578) and the American Society for Experimental Pathology April 1 1938.

² This investigation was aided by a grant from the Merck Fund for Surgical Research.

have been derived certain deductions concerning the mechanism by which a high carbohydrate, fat, or protein dietary affects the susceptibility of the liver to injury by chloroform

EXPERIMENTAL METHODS

Male albino rats were purchased at different ages and reared to a weight of 150 to 200 grams on a Steenbock (9) diet modified by substituting two per cent dried brewer's yeast for two per cent corn, thus constituted the "stock" diet.

The special diets (Table I), varying in carbohydrate, protein, and fat, were fed for a period of two to four weeks following the "stock" ration. In the groups of animals, designated VII-s in Table II, Diet VII was followed by an exclusive sucrose feeding for seven days. The result of this procedure was an animal whose liver contained both a high fat content as well as a high glycogen content, provided the rats ate sufficient of the sucrose offered. Group 8, Table II, while on Diet VII received, daily for nine days, about 160 mgm. of added choline in the form of choline chloride, in part mixed with the food and partially administered in solution, *per os*, also for the purpose of obtaining a higher concentration of hepatic glycogen and lower fatty-acids than was usually present on this diet. Groups 16 and 17

(Table II) received no food but were permitted water or the period of time designated in the Table. To the rats in Group 17, 100 mgm of choline (as choline chloride) in solution was administered, *per os*, daily for three days preceding the withdrawal of food, and also during the period of starvation.

The chloroform, for anesthesia, was volatilized in a Gwathmay three chamber volatilizing apparatus connected to a glass manifold leading to five percolator jars, each large enough to hold one rat. Clamps regulated the flow of the chloroform-air mixture led into each

chamber. A by-pass, for air alone, led a constant supply of air to each jar. Thus each rat was maintained in a uniform state of anesthesia at a level at which there was complete absence of movement. The depth of anesthesia was best judged by the movements of the tactile hairs (whiskers), the movement of which becomes slower the deeper the anesthesia. The period of anesthesia was uniformly one hour.

Twenty-four hours (except where noted) after the anesthesia with chloroform, about 50 mgm of sodium amytal was administered intraperitoneally. The abdomen was opened and, very rapidly, the caudate lobe of the liver was removed and placed in alcohol, later to be treated with Best's carmine stain, and a portion of the posterior lobule of the right lobe was boiled in a weighed amount of potassium hydroxide and later reweighed for glycogen determination. The remaining lobes were then removed, freed of extraneous tissue, and excess blood allowed to drain out. The left lobe (about 25 to 33 grams) was used for lipid analyses, the median lobe for solids, and the remainder of the right lobe was fixed in formalin for Sudan IV and hematoxylin and eosin staining. Glycogen was determined by the method of Good, Kramer, and Somogyi (10), using the Shaffer-Hartman reagent Number 2 (11) for the determination of glucose after hydrolysis. Total fatty acids were determined on the alcohol-ether extract of the liver by Long's (12) modification of the Stoddard and Drury method (13). Liver solids were obtained by drying the sample at 100° C. for 36 hours. Precisely the same histological and chemical procedures were employed with the normal control rats.

The criteria employed in the histological examination of the tissues were as follows. By the term degeneration is meant that, to a varying degree, the hepatic cells have undergone considerable swelling often to several times their normal size. The cytoplasm has a hyaline appearance and does not stain sharply with hematoxylin.

TABLE I
Composition of diets

	Percentage composition						Apportionment of calories			
	Diet I	Diet II	Diet III	Diet VI	Diet VII	Diet VIII	Diet number	Protein	Carbohydrate	Fat
								<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Casein†	16.0	28.2	20.0	81.2	9.6	9.0	I	17	83	
Sucrose	77.5		48.3		42.5	79.9	II	17		83
Crisco		60.3	21.8	7.2	38.7		III	17	41	42
Salt mixture‡	3.1	5.3	4.8	4.0	3.6	2.8	VI	83.3		16.7
Vegex*	3.2	5.9	4.9	7.4	5.3	7.2	VII	6.9	30.4	62.7
Oleum percomorphum‡	0.19	0.33	0.24	0.20	0.29	0.27	VIII	10.1	89.9	
Agar						0.89				
Calories per 10 grams	38.3	67.6	48.3	40.0	57.4	36.5				

* Kindly supplied by the Vitamin Food Company, New York City.

† Casein Number 453, Casein Manufacturing Company of America, New York City, extracted thoroughly with alcohol and ether.

‡ Salt mixture 185, McCollum, E. V. and Simonds, N. J. Biol. Chem., 1917, 32, 191.

§ Kindly supplied by Mead, Johnson and Company, Evansville, Indiana.

TABLE II

Diet composition of liver and hepatic damage 24 hours after chloroform anesthesia

Group number	Diet	Control Unanesthetized			Experimental Anesthetized						Remarks
		Number of rats	Average glycogen*	Average fatty acid†	Number of rats	Average glycogen*	Average fatty acid†	Number showing degeneration	Number with necrosis	Total damaged	
			per cent	per cent		per cent	per cent			per cent	
1	Stock	19	5.6	8.6	42	2.5	12.1	2	2	10	
2	I	14	5.5	9.1	22	1.8	11.0	2	0	9	
3	VI	5	2.4	11.4	10	1.9	16.3	3	0	30	
4	III	9	5.5	13.2	22	2.1	14.1	4	4	36	
5	II	4	1.2	17.0	8	0.4	13.7	3	2	63	Killed 48 hours
6	II	2	1.5	20.7	8	0.9	24.1	4	3	88	
7	VIII	2	7.0	21.7	2	0.3	39.0	0	2	100	
8	VII-c	3	5.0	23.4	7	1.4	29.9	1	5	86	Given choline chloride per os
9	II	3	1.4	24.3	6	1.2	21.3	3	3	100	
10	VII-s	4	8.3	24.5	8	1.0	24.2	0	8	100	Fed only sucrose 7 days
11	II	4	3.1	26.9	10	1.6	22.4	4	5	90	
12	VII	8	5.4	38.4	6	0.2	39.0	0	6	100	
13	VII-s	3	0.9	53.6	7	0.4	49.3	0	7	100	Fed only sucrose 7 days. Did not eat well
14	VII	4	2.7	56.9	13	0.4	59.8	1	12	100	
15	VII-s	4	3.5	62.4	6	1.7	70.4	0	6	100	Fed only sucrose 7 days
16	No food for 24 hours	4	0.12	14.5	10	0.49	16.8	0	10	100	Stock diet before starvation No food 24 hours after anesthesia
	No food for 48 hours	4	0.59	15.0							
17	No food for 24 hours	4	0.10	13.8	10	0.21	14.1	1	8	90	Choline for 3 days previous and during starvation
	No food for 48 hours	4	0.45	12.6							

* Glycogen is expressed as percentage of the wet weight of the liver

† Fatty acids are expressed as percentage of the dry weight of the liver

and eosin. The cell outlines are, in the main discrete, but they are, at times broken, so that the boundaries between the cells may not be sharply defined. The nuclei are often pyknotic. The appearance is that which one would expect if groups of cells had become markedly edematous, in the midst of others which appeared to be normal.

Where necrosis is reported the nuclei are greatly distorted or destroyed. The cell outlines have disappeared and the necrotic zone is in a state of complete disorganization. Obviously necrosis and degeneration frequently exist together. In many instances the necrosis is so extensive that the degree of associated degeneration can not be estimated with any degree of accuracy.

In the sections from livers in which fat has completely infiltrated the liver cells, it is at times difficult to state the exact extent of the necrosis. If no normal liver cells are present, and the cells between massive areas of fatty infiltration are necrotic, it is assumed that extensive necrosis is present. This is especially the case where the outlines of the fatty infiltrated cells are in part destroyed and the nuclei which are pushed to the side by the fat

droplets have undergone extensive change or been destroyed.

The histological abnormalities are reported as degeneration, when this alone was found as necrosis even though degeneration was also present. No attempt is made to indicate the extent of the particular degree of damage.

EXPERIMENTAL

By altering the diet (Table I) groups of rats were obtained whose hepatic lipid varied over a wide range (from 8.6 to 62.4 per cent of fatty acids by dry weight), and in which the glycogen of the liver was high or low at any given level of lipid concentration. At comparable fat levels the glycogen concentrations in the livers varied by 200 to 600 per cent. The protein content of the rations varied considerably, above and below that usually offered to growing rats. Furthermore, the dietaries have been so far as is possible, de-

vised so that data can be compiled (Table II), from which the rôle of each of the foodstuffs, and its resulting liver constituent, can be evaluated without being pertinently affected by the others.

In Table II the various groups of rats which had received the diets in Table I have been arranged in an ascending order of the average percentage content of fatty acids in their livers. In Table III (Compilation 1) the data have been apportioned into three groups in which the initial average concentration of fatty acid was low (10.5 per cent), at a medium level for our series (24.1 per cent) and high (49.7 per cent). It will be noted that the average initial concentration of glycogen in the livers of these groups did not differ greatly, namely, 4.9, 4.5, and 3.7 per cent respectively. The percentage of rats whose livers showed damage was 21 per cent in the low fat groups, 92 per cent at the medium, and 100 per cent at the very high level, livers the cells of which exhibited only degenerative changes are included, as well as those showing necrosis. The incidence of necrosis in the three groups was 8 per cent, 63 per cent, and 97 per cent. The incidence and the severity of the damage to the hepatic cells twenty-four hours following one hour of chloroform anesthesia increased progressively with an increase in the concentration of fatty acids in the liver. This is also apparent in the compilation of the individual groups of rats in Table II.

A further analysis of the data consisted of a division into groups with an initially high and low concentration of hepatic glycogen, in an effort to reveal the possible effect of this constituent of the liver in affecting its susceptibility to injury by chloroform. The grouping is somewhat arbitrary, but, we believe, fair. A concentration of glycogen of 5.0 per cent or above has been considered high and concentrations less than 5.0 per cent as low. In our experience in the rat, an average of 5.5 per cent of hepatic glycogen results from balanced diets high in carbohydrate, as illustrated by our "stock" ration and Diet I.

The first analysis (Table III, Compilation 2) of the results, on this basis, includes all of the data in Table II. The average initial concentrations of glycogen are 5.7 per cent for the high groups, and 2.2 per cent for the low. Anesthetization with chloroform in the former group (109 rats) was followed by degeneration or

necrosis of the liver in 33 per cent of the rats. In the second group (68 rats) with lower hepatic glycogen, 82 per cent showed hepatic injury. Of the total percentage exhibiting hepatic cellular damage, necrosis was found in 25 per cent of the livers with high initial (before anesthesia) glycogen and 56 per cent with the low. Conclusions from these results are invalidated by the fact that the average fatty acid concentration in the group high in glycogen was 15.7 per cent, whereas it was 34.0 per cent at the lower level of glycogen. Since the previous analysis of the data showed an increase in liver injury with increased lipid concentration, it became necessary to select from the data groupings in which the initial average fatty acid concentration was about the same. This was accomplished by eliminating the groups with very high and low concentrations of hepatic fatty acids before anesthesia, thus restricting the range between 11 and 27 per cent. Two groups are thus obtained, one with a hepatic glycogen of 6.2 per cent and with 18.4 per cent of fatty acids, and another with an average of 2.0 per cent glycogen and 19.3 per cent of fatty acids. In this arrangement of the data, the 39 rats high in glycogen showed a total incidence of hepatic injury of 62 per cent (13 per cent degeneration and 49 per cent necrosis), and at the lower level of glycogen (42 rats) 71 per cent of the livers presented histological evidence of cellular abnormalities (40 per cent degeneration and 31 per cent necrosis). These figures fail to show that a high concentration of hepatic glycogen, *per se*, confers a protection against the hepatotoxic action of chloroform in rats with the same medium concentration of fatty acids. In fact the severity of the lesions in the high glycogen group is slightly greater than in the low.

In evaluating the adverse effect of a high hepatic lipid content and the advantage, if any, of a high glycogen content the data as a whole have been arranged graphically. In Figure 1 the percentage incidence of liver damage in all of the rats in Table II has been plotted along the abscissa, and both the hepatic fatty acid concentration and the glycogen as ordinates. The fatty acid concentrations group themselves very closely along a straight line which slopes upward with the increase in the percentage of injured livers. This indicates a progressive additional incidence of

TABLE III
Compilation of data from Table II

Group numbers	Experimental state	Control			Experimental Apportionment of liver damage					
		Number of rats	Average liver glycogen	Average liver fatty acids	Number of rats	Degeneration		Necrosis		Total damaged
			per cent	per cent		number	per cent	number	per cent	per cent
COMPILATION 1 LIVER DAMAGE AT ASCENDING FAT LEVELS										
1 to 5 (inc.)	Low fat	51	4.9	10.5	104	14	13	8	8	21
6 to 11 (inc.)	Medium fat	18	4.5	24.1	41	12	29	26	63	92
12 to 15 (inc.)	High fat	19	3.7	49.7	32	1	3	31	97	100
COMPILATION 2 LIVER DAMAGE AT HIGH AND LOW GLYCOGEN LEVELS										
1 2 4 7 8 10 12	High glycogen	59	5.7	15.7	109	9	8	27	25	33
3 5 6 9 11 13 14 15	Low glycogen	29	2.2	34.0	68	18	26	38	56	82
4 7 8 10	High glycogen	18	6.2	18.4	39	5	13	19	49	62
3 5 6 9 11	Low glycogen	18	2.0	19.3	42	17	40	13	31	71
COMPILATION 3 LIVER DAMAGE ON HIGH AND LOW PROTEIN DIETS										
1 2 3 4 5 6 9 11	High protein diet	60	4.5	12.6	128	25	19	19	15	34
7 8, 10 12 13 14 15	Low protein diet	28	4.7	41.3	49	2	4	46	94	98
5 6 9 11	High protein diet	13	1.9	22.3	32	14	43	13	41	84
7 8 10	Low protein diet	9	6.9	23.5	17	1	6	15	88	94
COMPILATION 4 LIVER DAMAGE AT ASCENDING FAT LEVELS HIGH PROTEIN DIETS										
1 to 2 (inc.)	Low fat	33	5.6	8.8	64	4	6	2	3	9
3 to 5 (inc.)	Medium fat	18	3.7	13.5	40	10	25	6	15	40
6 9 11	High fat	9	2.2	24.7	24	11	46	11	46	92
COMPILATION 5 LIVER DAMAGE AT HIGH AND LOW GLYCOGEN LEVELS HIGH PROTEIN DIETS										
4	High glycogen	9	5.5	13.2	22	4	18	4	18	36
3 5	Low glycogen	9	1.9	13.9	18	6	33	2	11	44
COMPILATION 6 LIVER DAMAGE DURING STARVATION										
16 17	No food	8	0.1	14.2	20	1	5	18	90	95
4 5	II III	13	4.2	14.4	30	7	23	6	20	43

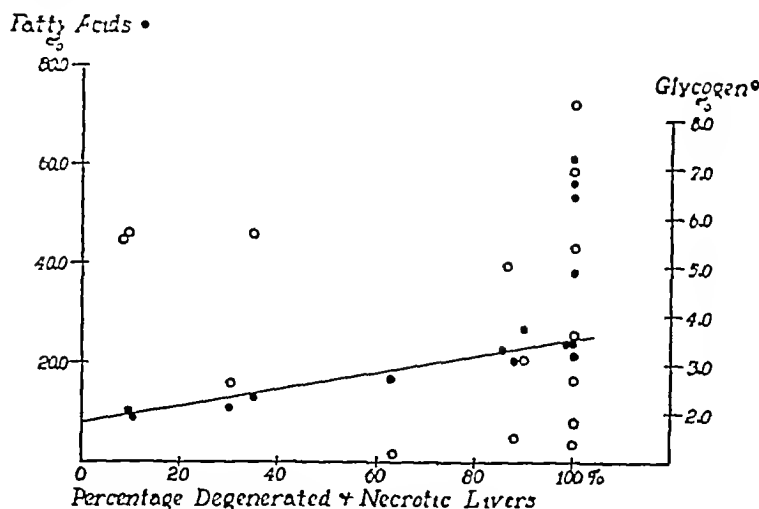


FIG 1 CHLOROFORM INJURY TO LIVER IN RELATION TO LIVER LIPID AND GLYCOGEN

damage to the liver as the hepatic lipid is increased. No similar relationship exists between the hepatic glycogen and the incidence of hepatic damage. The glycogen, therefore, exerted no apparent influence upon the susceptibility of the liver to the toxic effects of the chloroform. When the fatty-acid content reaches about 25 per cent all of the livers exhibit, to some degree, histological evidence of damage.

The various groups of rats are next apportioned (Table III, Compilation 3) into those with relatively high and low content of protein in their rations. Diets which derived 17 per cent or more of their total caloric value from protein were considered to be high in that foodstuff. This included the "stock" ration and Diets I, II, III, and VI. Seventeen per cent of the calories from protein is ample for the requirements of young growing rats. The diets low in protein contained but 10 per cent or less (Diets VII and VIII) of their calories as protein. This is distinctly less than is necessary to maintain good growth in the young rat. On this basis the data in Table II have been analyzed to determine the effect of the protein content of the food upon the resistance of the liver to the effects of chloroform.

Two analyses of the data have been made. The first includes all of the rats in Table II. The hepatic fatty acid concentrations, in the two groups so obtained, differ so greatly (12.6 and 41.3 per cent) that no conclusions as to the action of the

protein would be justifiable. It was necessary, therefore, to select groups of rats with comparable concentrations of hepatic lipid. This was accomplished by utilizing only those groups of rats with hepatic fatty acid concentrations ranging between 17 and 27 per cent. The average initial fatty acid concentration in the control rats, which received no chloroform, was 22.3 per cent in the animals which received a high protein diet, and 23.5 per cent in those whose rations were low in calories from protein. Of the 32 rats on the higher protein intake, the livers of 84 per cent were histologically abnormal following chloroform anesthesia. Hepatic cellular injury occurred in 94 per cent of the 17 rats on the diet low in protein. This difference is of doubtful significance. The marked and significant difference is to be found in the severity of the cellular changes. In the high protein group, areas of hepatic cellular necrosis were found in but 41 per cent of the rats, while in those which received a ration low in protein, necrosis was present in 88 per cent of the livers. It would appear that, although a high protein diet may not markedly affect the total incidence of hepatic damage due to chloroform, it does decidedly reduce the degree of the injury. This protective action of a diet high in protein reveals itself in animals whose livers contained a rather high lipid content (22 per cent). The average hepatic glycogen concentration in the animals on the higher protein intake was 1.9 per cent,

and 6.9 per cent on the lower. Therefore, the low protein animals had whatever advantage may accrue from a high hepatic glycogen content. Nevertheless, the high content of hepatic glycogen failed to exert a beneficial action of sufficient degree to become apparent in the presence of the detrimental effect of the low intake of protein and the same high hepatic lipid.

The slightly lesser percentage of necrosis in the rats with low hepatic glycogen (20 per cent) in the second grouping of Compilation 2 (Groups 3, 5, 6, 9, and 11) is probably due to the fact that the rations of these rats were all high in protein calories.

Inasmuch as the data show that dietary protein exerts a protective action against the toxic effects of chloroform upon the liver it becomes necessary to re-examine the data bearing upon the action of a high hepatic lipid or glycogen content, in the analysis of which this factor was not taken into account. The smooth slope of the line which passes through the fatty acid concentrations in Figure 1 would indicate that other factors influenced the susceptibility of the liver to injury to a lesser degree than did the hepatic lipid.

Additional evidence will be presented in which the factor of the protein is controlled. In Compilation 4 of Table III all of the data were derived from dietaries in which the protein was high. It comprised 17 per cent of the total caloric value of each unit amount of the ration, except in one group of rats (Number 3 Diet VI Table II) in which it was 83 per cent. In substantiation of the previous analysis of the data the incidence and severity of liver damage increased with an increase in the hepatic fatty acid concentration. Although the hepatic glycogen concentrations are not the same in this compilation none are very low and no evidence in these experiments indicates that this factor is of importance.

The influence of the hepatic glycogen content is also again analyzed in groups of rats which were fed rations comparable with respect to protein (Compilation 5 Table III). The total incidence of histological abnormality was 36 per cent in the rats with high glycogen (5.5 per cent) and 44 per cent in the low (1.9 per cent). Necrosis was found in 18 per cent of the livers with the

high glycogen content and 11 per cent in those with the low. These differences are of no significance. The average initial hepatic fatty acid concentration, namely 13 to 14 per cent, is considerably less than in the previous compilation (Number 2), consequently the damage from the chloroform was not so great. Therefore, any protective action of the hepatic glycogen should have a better opportunity to reveal itself. Despite this fact, and with the same high level of protein in the food, the glycogen content of the liver failed to show any protective action.

The effect of withdrawal of all food, except water for twenty-four hours preceding the administration of the chloroform was determined. This length of time without food has a profound effect in depleting the hepatic glycogen of the rat. In the two groups of control animals (Numbers 16 and 17 Table II) it was reduced to about 0.1 per cent. The anesthetized rats were offered no food during the twenty four hours following the anesthesia, when they were sacrificed. Hence, the final concentrations of hepatic glycogen and fatty acids are to be compared with those in the control animals starved for forty-eight hours. To one group of rats (Number 17) choline chloride was administered in an attempt to prevent the increase in hepatic lipid which attends starvation. Since this procedure was not successful, the two groups are analyzed together (Compilation 6 Table III).

The incidence of hepatic cellular injury in the starved rats is compared with that which was found in fed animals with the same initial hepatic fatty acid concentration but inevitably much higher glycogen levels (Groups 4 and 5, Table II). Ninety five per cent of the livers of the starved groups (20 rats) were histologically abnormal in 90 per cent areas of cellular necrosis were found. The fed group (30 rats) showed but 43 per cent of damaged livers with 20 per cent necrotic. The livers of the starved rats, therefore, were more susceptible to the effects of chloroform than those of the fed animal with the same low concentration (14 per cent) of hepatic fatty acids. The extent and severity of the damage to the liver of the starved rat is almost maximal and nearly equivalent to that in a fed animal with the highest (49.7 per cent) concentration of hepatic lipid.

DISCUSSION

Regardless of the reasons for the toxic action of chloroform upon the cells of the liver, the evidence presented shows that the incidence and degree of the injury increases with the concentration of lipid in the liver. A high fat intake increases the amount of fat deposited in the liver. Hence a causal relationship is established between our results and the previous finding, that a high fat diet increases the susceptibility of the liver to injury by chloroform (3, 4, 5), and, according to Davis (14), also by carbon tetrachloride. The data which are presented here serve as a firmer basis for the following proposed explanation of the mechanism of this phenomenon.

A rational explanation of the greatly increased susceptibility of a fatty liver to damage by chloroform was suggested by Wells in 1908 (8b, 8c). The fact that, clinically, chloroform seemed particularly to affect fatty livers (15), added to the well known solubility of chloroform in fats, led Wells to state "It would seem probable therefore, that a fatty liver would abstract much more chloroform from the blood than a normal liver and that the chloroform would thus act more strongly and for a longer time on the protoplasm of the fatty liver cells than it would in a normal liver." Opie and Alford (3b) accepted this explanation as probable, and pointed out that necrosis by chloroform occurred around the central vein in the lobule of the liver, where fat is deposited most abundantly.

The following clarification of the Wells hypothesis is suggested. The fat within the cells of the liver acts as a reservoir for the chloroform, therefore, a concentration sufficient to produce injury will be maintained during a prolonged period of desaturation. Final proof of this hypothesis will rest upon the chemical determination, following anesthesia, of the relative amounts of chloroform in livers with different contents of lipid. On "*a priori*" grounds the high distribution coefficient of chloroform between fat and water would make it quite certain that adequate chemical methods would reveal concentrations of chloroform corresponding to the amount of lipid in the hepatic cells.

The data reveal no evidence that the level of the hepatic glycogen, *per se*, at the time of anes-

thesia, influences the toxic action of chloroform. No plausible theory has been advanced to explain the protection conferred upon the liver by a high carbohydrate diet. The hypotheses which have been advanced to explain this protection all imply that it is due to the abundant and ready supply of energy made available by the increased deposits of hepatic glycogen which result from the diet.

The work and concepts of Rosenfeld (7) led to the suggestion that the administration of glucose might be of value in the prevention and treatment of necrosis of the liver by chloroform used for anesthesia (16).

He (7a, 7d) found that, in a variety of physiological and abnormal conditions, including poisoning by chloroform, in which the hepatic glycogen stores are nearly exhausted, there results an infiltration of fat into the liver. This occurs, according to Rosenfeld, because in the absence of glycogen, lipid, transported from the fat depots to the liver, accumulates there, since it cannot be burned unless carbohydrate is available and oxidized simultaneously. From these and other concepts, Rosenfeld (7b) concluded that, if the animal receives carbohydrate, the energy needs of the liver, increased as a defense against a noxious substance such as chloroform, will be satisfied, the liver will be protected, and hepatic fatty-infiltration prevented. Rosenfeld (7d) stated that the detoxifying power ("*entgiftenden Fähigkeiten*") of the liver is the greater the richer its content of carbohydrate.

Opie and Alford (3b) suggested that the necrosis produced by chloroform, phosphorus, and similar substances is perhaps the anatomical expression of advanced disintegration of body protein. Hence, carbohydrate may be of value in limiting the necrosis due to chloroform by exerting its recognized function of sparing body protein.

The implicit assumption in all previous concepts is that the depletion of hepatic glycogen by chloroform is due to an increased combustion, inferentially in the liver. An increased glycogenolysis does occur in the liver of both the dog (2) and the rat following anesthesia with chloroform, with the result that the stores of hepatic glycogen are reduced or almost depleted, an hyperglycemia is an invariable accompaniment. The behavior of

the blood sugar in relation to the glycogen stores in the liver does not suggest that utilization of glucose is proceeding at a greatly heightened rate the sugar in the blood may be above its pre-anesthetic level for twenty four hours, even though the hepatic glycogen is almost depleted at the end of this period of time (2). This would seem to be best explained by an inability of the hepatic cells to retain glycogen, rather than by the needs of a heightened metabolic activity of the hepatic cells as suggested by Rosenfeld (7b). The existence of a deranged capacity of the liver to deal with carbohydrate is supported by the finding (2) that glucose administered by stomach tube twenty-four hours following the anesthesia results in less deposition of hepatic glycogen than in the normal dog.

The same type of reasoning may be applied to the accumulation of lipid in the liver as a consequence of chloroform inhalation insofar as it is dependent upon carbohydrate utilization by the liver. In the dog it has been found that fat begins to accumulate in the liver at the end of a one or two-hour period of anesthesia with chloroform at a time when considerable glycogen is still present in the liver (2). Therefore the early fatty infiltration during and immediately following the anesthesia cannot be explained solely on the basis of a deficiency of carbohydrate to burn it, as claimed by Rosenfeld (7d 7e) if the glucose available in the hepatic glycogen is capable of combustion. For the same reason the initiation of the transport of lipid to the liver by the blood from the fat depots in this early period cannot be due entirely to a depletion of hepatic glycogen. Analysis of the data in this paper shows no greater infiltration of lipid into the liver of the rats with initially low than in those with high hepatic glycogen.

The hypotheses which assume that glycogen *per se* is effective in protecting the liver against the action of chloroform receive no support either in these considerations of the behavior of the hepatic glycogen and lipid and blood sugar or in the data on the incidence and severity of damage to the liver with high or low content of glycogen.

It would seem that the explanation of the protective action of a high carbohydrate diet must lie in some concomitant effects produced by large deposits of hepatic glycogen. As already stated

Rosenfeld (7a 7b) found that, under many conditions in the body depletion of hepatic glycogen is followed by an increase in fat in that organ and *vice versa*. Evidence already presented (2), and instances in this paper of a coexistence in the liver of a high concentration of glycogen with a high lipid content (Table II, Groups 7 8 10, and 12) and other data to be published later, show that the reciprocal relationship of Rosenfeld (7d) is not an invariable rule. However, we have found in both the dog and the rat that a diet rich in carbohydrate, and, in the rat at least, adequate in protein, produces livers which contain maximal concentrations of glycogen but minimal concentrations of fatty acids. The "stock" diet and Diet 1, Table I are examples of such dietaries. The concentration of hepatic glycogen and lipid of normal rats fed with these rations is shown in Table II. Our experience has shown that in the normal dog or rat a high carbohydrate intake, in an otherwise balanced dietary will cause the hepatic lipid if high, gradually to diminish in amount.

The susceptibility of the liver to injury by chloroform is markedly enhanced by the presence of small increments of fat (Compilation 4 Table II), hence it would seem plausible to conclude, since glycogen as such is ineffective even at low levels of hepatic lipid (Compilation 5, Table II), that the protective action of a high carbohydrate diet is chiefly due to its effect in reducing hepatic lipid. This hypothesis would satisfactorily account for the reported failure to obtain protection from injury by chloroform when glucose is administered during the anesthesia (4).

Comparisons of the relative protection offered by a carbohydrate and a high protein diet are futile unless the fat contents of the livers are known. In the final analysis, the comparative protective values to the liver of the foodstuffs against chloroform resolves itself into the positive action of dietary protein *versus* the increased susceptibility due to hepatic lipid.

In view of the high degree of protection which an abundant protein dietary confers upon the liver, it must be conceded that carbohydrate may also be of value under conditions in which it can act as a sparer of protein.

In contrast to this indirect action of carbohydrate in decreasing the damaging effects of chloro-

form upon the hepatic cells, the protection afforded by protein would appear to be more direct and related to some intrinsic value of the protein itself. Pertinent, in this respect, is the finding (Compilation 3, Table II) that a previous high protein diet protects the liver against necrosis by chloroform at a high level of hepatic fatty acids (22 per cent). This would indicate that the protein factor, whatever it may be, is a very potent one, for it can counteract, to some extent, the untoward effects of a high hepatic lipid, which means that it is effective under conditions in which a very severe injury by chloroform usually occurs.

Moise and Smith (5a) also found that the extent of the necrosis is less in rats on a high protein than on other diets. It may be significant that in the present experiments, as well as in those of Moise and Smith, casein constituted the protein which was fed. Davis and Whipple (4) also reported that, in the dog, skim milk or casein are very protective, while skeletal and cardiac muscle are less so. Opie and Alford (3b) found a greater toxicity of chloroform in rats fed solely meat than in those given a high carbohydrate diet, and concluded that protein is less protective than carbohydrate.

Although the protein action is probably a direct one, certain facts at hand, soon to be published, show that the level of hepatic lipid and glycogen are markedly affected by the amount of protein in the diet. Best, Channon, and their co-workers (17) have reported, and we have confirmation of their finding, that a high protein dietary is conducive to a lesser deposition of liver lipid (*ic*, Diet VI, Table II), than is a low protein intake (*ic*, Diet VIII, Table II). Here, then, as in the case of a high carbohydrate diet, the level of protein in the dietary may affect the stores of hepatic lipid. It is also apparent from the results obtained from feeding Diet VIII (Table II) that a low protein intake may lead to an increase of lipid in the liver, despite the presence of an excess of carbohydrate calories. This factor is not involved in the discussion in this paper for the comparisons of the high and low protein diets are for rats with about the same concentration of hepatic fatty acids.

The mechanism by which the protein of the diet aids the liver in resisting the necrotic action of chloroform, with our present information, can

only be a subject of conjecture, although this problem is being pursued at present in our laboratories.

The question of protein storage in the body, on a high protein diet, becomes of particular importance in relation to the protection conferred by such a diet, especially if we assume that it is due to protein, *per se*. It is of interest that investigators in the field of protein storage have invariably directed their efforts to the liver as the most probable storehouse for protein. Recently the problem has been investigated by Luck (18). He found that in the rat, on a high protein intake, in accordance with previous results, the total protein content of the liver, as well as the amount per unit of weight is increased above that found on a low protein diet. Addis, Poo, and Lew (19) in a study of the loss of protein from various organs and tissues of the body during a fast found that the liver loses so much more of its original protein content than any other organ, that it "suggests that it may be a depot for stored protein and that this special sort of protein may be used during fasting in much the same manner as glycogen is used during a fast."

It would seem that there is some basis for believing that a high protein diet previous to liver injury by chloroform may make available stores of protein within the body. This protein, stored or elaborated into hepatic or other body tissue, may serve to protect the cells or to replenish a structure which is being attacked. Which of these alternative mechanisms is operative is impossible to decide at present. It may, however, be supposed that either the available stores of protein prevent or ameliorate the destructive action of the chloroform or that the damage which occurs is rapidly repaired and does not proceed to necrosis. It will be recalled that the data show that the main result of a high, as compared to a low, protein diet is to decrease the incidence of hepatic necrosis. The two processes of damage and repair might be conceived as proceeding at the same time.

The greatly increased susceptibility of the liver of the starved rat to necrosis by chloroform, evident in the data we have presented, is in accord with the previous findings of Davis and Whipple (4) and of Smith and Moise (5b). Since the incidence and degree of the hepatic damage caused

by chloroform is much greater in the rat which has received no food for twenty four hours than that in the fed animal with the same low concentration (14 per cent) of hepatic fatty acids the greater susceptibility of the former animal to hepatic injury is probably not due to differences in the lipid content of the liver. The evidence already presented in this paper makes it very doubtful that the low hepatic glycogen, *per se* of the starved animal is the factor responsible for the greater vulnerability of the liver.

In view of these deductions and the importance of the protein factor it would seem that the most probable deficiency in the unfed animal, responsible for the increased susceptibility to injury is its stores of protein which have been shown to be diminished in the body tissues and especially in the liver when the rat is starved (19). This conclusion is in harmony with our finding of increased hepatic cellular damage in rats on low protein diets as compared to those which received a high protein ration.

The constituents of a balanced diet which by altering its composition confer protection upon the liver are the protein probably directly and the carbohydrate indirectly by conditioning the content of hepatic lipid. The starved animal enjoys to only a slight extent, or not at all, the advantages to be derived from either of these dietary factors. It would follow that if carbohydrate alone were fed, it may be protective to the liver, by virtue of its indirect action as a sparer of protein. This result would depend upon the length of time the carbohydrate is fed for as previously stated the absence of protein intake predisposes to fat deposition in the liver which might offset the advantage derived from a small amount of conserved protein.

The work of Davis, Hall, and Whipple (20) and Daft, Robschek, Robbins and Whipple (21) is of interest in this discussion. They produce evidence from which they conclude that in a starved dog the "protein split products" which usually appear in the urine in increased amounts following chloroform anesthesia may be partially 'conserved' by the administration of glucose, and are thus made available for regeneration, since they find under these conditions a more rapid regeneration of the liver cells than in a starved dog not given glucose. They make a sharp distinction

between this type of protein sparing action of carbohydrate namely a "conservation of protein split products" and the usual concept that carbohydrate, instead of protein is utilized for energy production, when glucose is given to a starved animal, and thus spares the protein 'at its source'. These findings constitute an excellent example of the protein sparing action of carbohydrate under conditions in which the protein is needed for building of new body tissue. Regardless of how the protein is spared or utilized, it emphasizes the importance of protein in the condition under discussion and the role which carbohydrate may have in its conservation.

It would be unwise to generalize our findings. One might expect to find that the destructive action of all fat soluble hepatotoxic agents would be affected by the amount of hepatic lipid. An adequate high carbohydrate diet, by virtue of its effect in lowering the hepatic lipid content might decrease the incidence and severity of the damage to the liver. A high protein diet might be protective against the necrotic action of hepatic poisons regardless of their fat solubility and carbohydrate administration, under conditions in which it spared protein might be consequently advantageous. Since the site and perhaps the mode of action of the various hepatotoxic substances may differ, it would seem advisable to examine each one in the light of the above stated possibilities.

lipid content of the liver which results from such a diet. Under certain conditions, i.e., inanition, administration of carbohydrate may very probably also protect the liver by virtue of its protein sparing action. There is no evidence that the depletion of hepatic glycogen by chloroform is associated with the metabolic requirements of the liver, but is probably a manifestation of an effect of the toxic agent.

4 A high protein diet, previous to the anesthesia with chloroform, markedly reduces the incidence of hepatic cellular necrosis, even in livers with a high lipid content, and, therefore, in the face of a severe attack by the chloroform. Regardless of whether this is due to a protection against injury in the first instance, or to a less intensive lesion due to a rapid regeneration, *pari passu* with the injury, it would seem to be best explained as being primarily the result of protein available to the liver as a result of the high protein intake.

5 Rats, starved for twenty-four hours prior to one hour of chloroform anesthesia, exhibit in almost all instances some degree of hepatic injury, largely necrosis. The incidence and degree of the damage is greater than in fed animals with the same content of hepatic lipid. Tentatively, it is suggested that the increased susceptibility of the starved rat is principally due to its depleted protein stores.

BIBLIOGRAPHY

- 1 Goldschmidt, S, Ravdin, I S, and Lucke, B, The protective action of oxygen against the necrotizing effect of certain anesthetics on the liver. *J Pharmacol and Exper Therap.*, 1937, 59, 1
- 2 Ravdin, I S, Vars, H. M, Goldschmidt, S, and Klingensmith, L, The effect of anesthesia on the blood sugar, the liver glycogen, and liver fat. *J Pharmacol and Exper Therap.*, 1938, 64, 111
- 3 (a) Opie, E L., and Alford, L. B, The influence of diet on hepatic necrosis and toxicity of chloroform. *J A. M. A.*, 1914, 62, 895
(b) *Ibid.* Diet and the hepatic lesions of chloroform, phosphorus, or alcohol. *J Exper Med.*, 1915, 21, 1
Diet and the nephritis caused by potassium chromate, uranium nitrate, or chloroform. *J Exper Med.*, 1915, 21, 21
- 4 Davis, N C., and Whipple, G H, The influence of fasting and various diets on the liver injury effected by chloroform anaesthesia. *Arch. Int. Med.*, 1919, 23, 612
- 5 (a) Moise, T S, and Smith, A H, The regeneration of liver tissue on various adequate diets. *J Exper Med*, 1924, 40, 13
(b) Smith, A H, and Moise, T S, The regeneration of liver tissue during nutrition on inadequate diets and fasting. *J Exper Med*, 1924, 40, 209
- 6 Minot, A S, and Cutler, J T, Guanidine retention and calcium reserve as antagonistic factors in carbon tetrachloride and chloroform poisoning. *J Clin Invest.*, 1928, 6, 369
- Cutler, J T, The accumulation of guanidine in the blood following acute liver injury by carbon tetrachloride, chloroform, arsenic or phosphorus. *J Pharmacol and Exper Therap*, 1931, 41, 337
- 7 (a) Rosenfeld, G, Zur Lehre von der Fettwanderung. *Allgemeine Medicinische Zentral Zeitung*, 1900, No 89, 1051
(b) *Ibid.* Fettbildung. *Ergebn d. Physiol.*, 1903, 2, 50
(c) *Ibid.* Der Process der Verfettung. *Berl. Klin. Wchnschr.*, 1904, 41, 587
(d) *Ibid.* Fett und Kohlenhydrate. *Berl. Klin. Wchnschr.*, 1906, 43, 978
(e) *Ibid.* Eiweiss Körper und Leberverfettung. *Berl. Klin. Wchnschr.*, 1910, 47, 1268.
- 8 (a) Wells, H G, Chemical Pathology. Saunders, Philadelphia, 1925, 5th ed., pp 20 and 481
(b) *Ibid.* Delayed chloroform poisoning and allied conditions. A note on the cause of the anatomic and clinical changes observed. *J A. M. A.*, 1906, 46, 341
(c) *Ibid.* Chloroform necrosis of the liver. *Arch. Int. Med.*, 1908, 1, 589
- 9 Steenbock, H, A satisfactory ration for stock rats. *Science*, 1923, 58, 449
- 10 Good, C. A, Kramer, H, and Somogyi, M, The determination of glycogen. *J Biol. Chem.*, 1933, 100, 485
- 11 Peters, J P, and Van Slyke, D D, Quantitative Clinical Chemistry. Vol II. Methods. Williams and Wilkins Co, Baltimore, 1932, p 466
- 12 Long, C. N H, Personal communication
- 13 Stoddard, J L, and Drury, P E, A titration method for blood fat. *J Biol. Chem.*, 1929, 84, 741
- 14 Davis, N C., The influence of diet upon the liver injury produced by carbon tetrachloride. *J Med. Res.*, 1924, 44, 601
- 15 Guthrie, L G, On the fatal effects of chloroform on children suffering from a peculiar condition of fatty liver. *Lancet*, 1903, 2, 10
- 16 Beddard, A P, A suggestion for treatment in delayed chloroform poisoning. *Lancet*, 1908, 1, 782.
- 17 Best, C. H, Huntsman, M E., and Ridout, J H, The "lipotropic" effect of protein. *Nature*, 1935, 135, 821
- Channon, H J, and Wilkinson, H, Protein and the dietary production of fatty livers. *Biochem. J.*, 1935, 29, 350

- Best, C. H., and Channon, H. J., The action of choline and other substances in the prevention and cure of fatty livers. *Biochem. J.* 1935 29 2651.
- Best, C. H., Grant, R., and Ridout J. H., The "lipotropic" effect of dietary protein. *J. Physiol.* 1936 86, 337
- 18 Luck, J. M., The question of protein storage. *J. Biol. Chem.*, 1936, 115, 491
- 19 Addis T., Poo, I. J., and Lew W., The quantities of protein lost by the various organs and tissues of the body during a fast. *J. Biol. Chem.* 1936, 115 111
- Protein loss from liver during a two day fast. *J. Biol. Chem.*, 1936 115 117
- 20 Davis N. C., Hall, C. C., and Whipple, G. H., The rapid construction of liver cell protein on a strict carbohydrate diet contrasted with fasting. Mechanism of protein sparing action of carbohydrate. *Arch. Int. Med.*, 1919 23, 689
- 21 Daft, F. S., Robscheit Robbins F. S., and Whipple G. H., Liver injury by chloroform nitrogen metabolism, and conservation. Liver function and hemoglobin production in anemia. *J. Biol. Chem.*, 1936 113, 391

THE NATURE OF LEUKEMIC BLOOD CELLS AS DETERMINED BY THEIR METABOLISM

By WALTER KEMPNER

(From the Department of Medicine Duke University School of Medicine Durham)

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The nature and etiology of leukemia are unknown (1). Some authors (2, 3, 4, 5, 6, 7, 8) are in favor of classifying the leukemias as malignant neoplasms of the blood forming organs because of the formation of leukocytes in unaccustomed organs, the new growth of leukemic tissue and the flooding of the blood stream with abnormal primitive cells. Others (9) prefer to consider leukemia as a type of benign tumor formation or tissue metaplasia or simply as an excessive unregulated production and outpouring of essentially normal young tissue cells. To solve the problem by means of morphological histology has proved impossible (10).

A new approach to this question was opened, however, by the methods of cellular physiology and this paper presents experimental results obtained by these methods, which, it seems to me, give an unambiguous and definite answer to the question of the nature of leukemic cells.

As Warburg (11) has found cells of benign, and to an even greater degree of malignant tumors differ fundamentally from normal cells in their energy supplying metabolic reactions. In all normal undamaged animal cells the magnitude of the oxidative metabolism is considerably greater than the magnitude of the glycolytic metabolism and, under aerobic conditions, no splitting of sugar into lactic acid (glycolysis) occurs. In the metabolism of benign tumor cells and even more of cancer cells the respiration-glycolysis ratio is disturbed the rate of glycolysis is high as compared to the rate of respiration and a great amount of lactic acid is formed under aerobic conditions. This aerobic glycolysis however, may have a different origin, it can be produced, for example, by poisoning the cells with cyanide, carbon monoxide, or arsenious acid by various injuries leading to the gradual death of the cell, or in the more sensitive organs simply by keeping the tissue in Ringer's solution instead of its own plasma. Aerobic glycolysis as such then is not specific for malignancy, but rather the fact that the cancer

cell can live and grow by its persisting glycolytic metabolism under aerobic and anaerobic conditions whereas aerobic glycolysis in injured cells is only a metabolic phase of their dying off. If, therefore under physiological experimental conditions the determination of the cell metabolism reveals aerobic glycolysis of zero, it is certain that the tissue examined is normal and not cancer tissue whereas the positive finding of aerobic glycolysis need not be specific for cancer cells but may characterize either cancer metabolism or the dying off of any tissue within or without the body.

Since the results of experiments on the metabolism of white blood cells are scattered through the literature and there is no critical survey on the suitability or inadequateness of the methods employed, it seems advisable to give a bibliographical review on this subject.

Grafe (12) examined the blood of leukemic patients and showed that white blood cells have a high oxidative metabolism.

Levene and Meyer (13) were the first to show that leukocytes have a high glycolytic activity. This was confirmed by Burger (14) for leukemic leukocytes.

Bakker (15) examined exudate leukocytes of rabbits, comparing their oxygen consumption and their glycolysis, and found a small rate of respiration together with high aerobic glycolysis. He concluded from these findings that white blood cells have cancer metabolism.

Fleischmann and Kubowitz (16) measured the metabolism of leukocytes from the blood of geese and from exudate of rabbits and found, like Bakker in both instances high aerobic glycolysis. But since the aerobic glycolysis was higher in the white cells from exudate than in those from blood, they concluded that aerobic glycolysis in leukocytes is not a proof of cancer metabolism but only a symptom of injury as leukocytes will die off more rapidly in exudates than within the blood vessels.

Fujita (17) examined white blood cells and bone marrow of rats, Barron and Harrop (18) leukocytes from peritoneal exudates of rabbits, Jackson, Parker and Glover (19) monocytes of rabbit's lung, Loebel Shorr and Richardson (23) monocytes of rabbit's peritoneum.

Common to all these experiments with mature animal leukocytes of various types, was the ambiguous result that the fermentative metabolism was high in comparison to the oxidative metabolism and that splitting of sugar into lactic acid took place under aerobic conditions. The original question remained unsolved, whether this me-

tabolism of leukocytes which all investigators agreed was different from the metabolism of normal cells, actually represented *cancer metabolism* or was *only due to injury or death of sensitive cells*

Regarding the metabolism of human leukocytes, Kempner and Peschel (22) examined mononuclear and polynuclear cells obtained from sterile blisters of the skin. They found the absolute metabolism figures considerably higher than those reported for the various animal leukocytes, but the type of metabolism as such was identical with that of the exudate leukocytes of animals: high glycolysis, relatively low respiration, and also *lactic acid formation under aerobic conditions*

Barron and Harrop (18) studied oxygen consumption and glycolysis in blood of patients with chronic leukemia and found *aerobic glycolysis present both in the blood of patients with lymphatic and with myeloid leukemia*. Unfortunately, the oxygen consumption was measured in the total absence of carbon dioxide (alkaline in the side bulb) and at a pH probably above 8 (pH determinations are not given), glycolysis was examined in N_2 instead of 5 per cent CO_2 /95 per cent N_2 . Furthermore, in the experiments with leukemic blood cells there are no figures given which indicate the amount of lactic acid formed by the cells but only the amount of sugar consumed, so that it is not possible to decide how much of the sugar consumed under aerobic conditions was used in respiration, how much for the formation of glycogen (30), and how much was actually split into lactic acid. On the basis of these sugar determinations the authors state that polynuclear leukocytes possess a higher aerobic and anaerobic glycolysis than mononuclear leukocytes, and calculating Warburg's so-called "excess of fermentation" ($U = Q_{N_2} - 2Q_{O_2}$) they conclude that *the metabolism type of lymphatic leukemia blood cells resembles that of normal cells, whereas myeloid leukemia leukocytes show the metabolism type of cancer tissue*. They further state that there is *no difference in the metabolism of mature and immature granulocytes* and of granulocytes from cases of leukocytosis or of myelogenous leukemia. Since the metabolism figures given in their paper are calculated per million cells, not per weight, it is difficult to compare them with the results of other investigators. For the sake of completeness, however, we have included them in Table I, assuming—very inaccurately—that the dry weight of 10 million white blood cells is equal to 1 mgm.

Glover, Daland, and Schmitz (20) made an extensive study of the metabolism of normal and leukemic leukocytes, determining the oxygen and sugar consumption in the blood of patients with lymphatic and myelogenous leukemia. They too, like Barron and Harrop (18), measured the oxygen consumption in the presence of alkaline, eliminating the physiological carbon dioxide content, and not at a physiological pH, but between 7.6 and 8.2. For the determination of glycolysis they did not use any lactic acid determinations but only sugar determinations, so that in this paper also it cannot be gathered how much of the sugar consumption is due to sugar oxidation, to the formation of glycogen from sugar, or to the

splitting of sugar into lactic acid. Oxygen and sugar consumption were not determined in the same medium, the rate of glycolysis was calculated by deducting from the total sugar consumption the amount of sugar oxidized assuming that all the oxygen consumed was used for the oxidation of sugar. In four cases of acute leukemia with for the most part *very primitive cells* (the authors say it has been impossible to determine exactly whether they were of lymphatic or myelogenous origin) they found the *aerobic glycolysis higher* than in cases of lymphatic and myelogenous leukemia with more mature cells, when using air to saturate the cell suspensions with oxygen. This, they state, was directly opposed to the results they obtained when the cell suspensions were exposed to 95 per cent oxygen. These conflicting results, however, are not due, as the authors think, to the difference between the effect of oxygen and that of air upon the cells. They only reveal the two other sources of error in the experiments under discussion. For, firstly, in the experiments in which the blood was saturated with air, the oxygen saturation (21 per cent) was evidently not sufficient to prevent the occurrence of completely anaerobic areas—especially since the blood was only submitted to "gentle agitation"—so that what was measured was not really the aerobic sugar consumption, but aerobic and anaerobic sugar consumption in undefined proportion, secondly, to the air used for saturation, no carbon dioxide was added, so that the experiment was done in the absence of carbon dioxide at an alkaline pH, whereas to the 95 per cent oxygen 5 per cent carbon dioxide was added, thus producing physiological conditions as to carbon dioxide concentration and pH. Determining the aerobic sugar consumption of the blood cells from five patients with myelogenous and five patients with lymphatic leukemia in this physiological milieu of 5 per cent CO_2 /95 per cent O_2 , Glover and his coworkers actually did find *aerobic glycolysis still present* in all of the five cases of myelogenous leukemia, but it *decreased with the immaturity of the cells*. Of the five cases of lymphatic leukemia, they found aerobic glycolysis present in three cases and absent in two, independent of the maturity or immaturity of the cells. Unfortunately, in this series of experiments, the only ones without fundamental errors in technique, only the sugar consumption and not the oxygen consumption was determined.

Schlossmann (21) and Peschel (1) examined blood cells from patients with lymphatic leukemia, determining oxygen consumption, aerobic and anaerobic lactic acid formation under physiological conditions manometrically with the Warburg technique. They were the first who were able to show that these immature lymphocytes—provided they are preserved from all injury and examined under physiological conditions—possess a purely oxidative metabolism and *no aerobic glycolysis* at all. From these findings Peschel concluded. Since mature and immature lymphocytes have a very different metabolism, in that the immature leukemic lymphocytes have a purely oxidative metabolism and do not show any formation of lactic acid under aerobic conditions, it is in the first place definitely proved that leukemic lymphocytes in regard

TABLE I

Summary of quantitative data on the metabolism of white blood cells (calculated for 1 mgm of dry weight of cells per hour)

Authors	Type of leukocytes	I Q_{O_2} Respiration (c.mm. oxygen consumed in air)	II Q_{O_2} Q_M Aerobic glycolysis (c.mm * lactic acid formed in air)	III Q_{N_2} Q_M Anaerobic glycolysis (c.mm * lactic acid formed in absence of oxygen)	IV Inhibition of lactic acid formation by air	V Ratio of aerobic gly- colysis to respiration II I	
Bakker 1927 (15)	Exudate leukocytes of rabbits	0.4	5.7	6.0	per cent 5	14.3	
Fleischmann and Kubowitz 1927 (16)	Exudate leukocytes of rabbits	4.5	14.0	23.3	40	3.10	
	Blood leukocytes of geese	4.9	1.8	11.6	85	0.37	
Fujita 1928 (17)	Bone marrow cells of rats	9.8	3.7	21.0	82	0.38	
	Blood leukocytes of rats	9.2	2.6	20.2	87	0.28	
Jackson Parker and Glover 1930 (19)	Monocytes of rabbits lungs	5.6	1.4†	2.7†	48	0.25	
Loebel, Schorr, and Richardson 1932 (23)	Monocytes of rabbits peri- toneum	2.8	3.0	4.9	39	1.07	
Barron and Harrop 1929 (18)	Chronic myelogenous leukemia	8.0	9.0†			1.12	
	Chronic lymphatic leukemia	4.9	2.0†			0.41	
Glover Daland and Schmitz 1930 (20)	Myelogenous leukemia	Immature	2.4	11.2† 2.8†	17.8†	84	4.66
		Mature	2.6	8.0† 17.8†	16.0†	0	3.08
	Lymphatic leukemia	Immature	2.4	11.2† 1.5†	15.9†	91	4.66
		Mature	5.8	5.2† 2.2†	17.2†	87	0.90
	Normal human blood leukocytes		7.1	8.8† 13.8†	13.8†	0	1.24
	Schlossmann 1930 (21)	Leukemic lymphocytes	12.0	0	21.9	100	0
Peschel 1930 (1)	Leukemic lymphocytes	5.8	0	11.1	100	0	
Kempner and Peschel 1930 (22)	Human exudate leukocytes	22.8	16.8	57.8	71	0.74	
Bossa (1937) (29)	Myelogenous leukemia	Chronic state	10.2	14.9	29.8	50	1.46
		Acute state	12.2	17.8	23.1	23	1.46
	Chronic lymphatic leukemia		13.3	0.9	17.8	95	0.07

* 1 c.mm. = 0.004 mgm. of lactic acid

† Lactic acid formation not determined manometrically nor chemically but assumed to be equal to sugar consumption

to their metabolism are not tumor cells but normal young tissue cells. Secondly the concept of a transition from normal lymphocytes to tumor cells two leukemic degenerated cells is excluded thirdly the aerobic glycolysis of mature lymphocytes is only a manifestation of their dying off in the blood stream or exudate. The signifi-

cance of Peschel's findings has been repeatedly discussed and emphasized, for instance in great detail in W. Fleischmann's review (24 see also 25) and by von Bergmann (26).

Soffer and Wintrobe (27) examined oxygen and sugar consumption in the blood of patients with mye-

ogenous and lymphatic leukemia. The proportion of myeloblasts in the leukocytes specimen examined varied between 4 and 33 per cent. Soffer and Wintrobe used the same methods as Barron and Harrop (18) and came to the same conclusions that the metabolism of the *blood of myeloid leukemia resembles that of cancer*, while that of *lymphatic leukemia* is more similar to that of *normal tissue*. The additional contributions of this paper are quantitative data which show that the unphysiological conditions of the method applied for examining the blood cells (absence of carbon dioxide, undefined pH, insufficient bicarbonate and sugar concentrations) injure the cells to such an extent that, as the authors state, a considerable and persistent decrease of the rate of respiration sets in at the very beginning of their experiment, so that after the first half hour, for example, the rate of respiration has dropped to only one-third of that measured in the first ten minutes. The second result of this work, viz., that the degree of this decrease of the respiration rate depends on the number of cells per volume of suspension fluid, indicates still better the detrimental effect of the unphysiological conditions enumerated above, for it is obvious that the rate of all changes in the composition of the suspension medium (sugar-, bicarbonate concentration, pH) will increase directly with the increase of the number of metabolizing cells present.

Horsters published a paper on comparative investigations on glycolysis of myeloid and lymphatic leukocytes (28). His procedure was to fill blood citrate suspensions in closed test tubes and allow them to stand for 1 to 2 days at room temperature, then in the leukocytes layer, diluted ten times with saline solution, sugar consumption was measured in 10-minute periods and lactic acid formation in 180-minute periods. The carbon dioxide concentration was zero, the weight of the blood cells was not given. The blood was saturated with air in a standing cylinder every ten minutes, but not shaken in the intervals, so that it cannot be told for what proportion of time the cells were under aerobic and anaerobic conditions respectively. The conclusions of this paper that suspensions of leukocytes of the myeloid type consume sugar relatively quickly in an atmosphere of air (38° C.) and form equivalent amounts of lactic acid, and that sugar consumption and lactic acid formation of suspensions of lymphocytes are relatively small, are similar to the statements of Barron and Harrop (18), Glover, Daland, and Schmitz (20), and Soffer and Wintrobe (27), although Horsters omitted mentioning these authors as well as the experiments of Peschel (1) and the review of Fleischmann (24).

The most recent study of the metabolism of leukemic cells was made by Bossa (29). He obtained leukocytes by brief centrifuging and suspended them in the serum of normal people. Oxygen consumption, aerobic and anaerobic glycolysis were determined by the Warburg methods. In the total number of white blood cells the differential proportion of myeloblasts varied from 1 to 40 per cent, that of lymphoblasts from 10 to 70 per cent. Bossa arrived at the same results as previous investi-

gators in all cases of *myelogenous leukemia*, whether of the acute or chronic type, he found a *high aerobic glycolysis* (Q_{O_2} 4.2 to 37.3). The average ratio of aerobic glycolysis/respiration in his cases of chronic myelogenous leukemia was exactly the same as in his cases of acute myelogenous leukemia. In leukemic lymphocytes he found the aerobic glycolysis either very small or zero. In spite of the high aerobic glycolysis of myelogenous cells, Bossa does not consider their metabolism as *signifying cancer metabolism but merely as a symptom*, as Peschel had done, of cell injury within or outside the body and therefore nothing but a transitional metabolic phase before the ultimate death of the cells.

The study of leukemic lymph nodes yielded the same ambiguous result (positive aerobic glycolysis) as that of leukemic blood cells. Victor and Potter (31) and Victor and Wintersteiner (32) examined the metabolism of lymph nodes of normal mice and of mice inoculated with different lines of transmissible lymphogenous leukemia, controlling age and genetic constitution. In all cases regardless of age, line, transfer number, and host conditions, they found *aerobic glycolysis*, which was usually even *greater in the leukemic than in the normal lymph nodes*. From the presence of aerobic glycolysis in all the lymph nodes, both leukemic and normal, the authors concluded that it was impossible to differentiate infections from neoplasms using cellular metabolism as a criterion.

Table I gives a summary of the quantitative data from the above mentioned experiments on the metabolism of normal and leukemic white blood cells. It must, however, be emphasized again that only part of these results were obtained by satisfactory methods and under physiological conditions. It is evident from the table that the *aerobic glycolysis-respiration ratio in granulocytes is always positive*. Calculated from the given figures (Column V) it varies in mature blood and exudate leukocytes between 0.25 and 3.1 (Bakker's figure, 14.3, excluded), in leukemic granulocytes between 1.1 and 4.7, in leukemic blood lymphocytes examined under physiological conditions it is zero. That means all "*normal*," i.e., *mature human and animal blood and exudate leukocytes, as well as the more immature granulocytes from all the patients with myelogenous leukemia, showed a lactic acid formation under aerobic conditions*, the absolute values varied from 1.4 to 17.8 mm of lactic acid formed by 1 mgm of leukocytes in 1 hour, whereas in the *immature leukemic lymphocytes, aerobic lactic acid formation did not occur*.

EXPERIMENTAL

In a large series of experiments on 40 cases of myelogenous and 15 cases of lymphatic leukemia, I have found, in complete agreement with other authors (1, 21) that leukemic lymphocytes do not show any aerobic lactic acid formation, that is, that they exhibit the type of metabolism characteristic of undamaged normal young cells.

The experimental approach to the question of

the nature of leukemic granulocytes proved to be complicated and difficult, since in most cases of myelogenous leukemia accurate measuring of the actual metabolism of the immature cells exclusively is not possible. For, if there is a large proportion of myeloblasts and the total white blood count is small, the metabolism of the myeloblasts is obscured by the respiration and splitting metabolism of the red blood cells. If, on the contrary the white blood count is high and the proportion of myeloblasts small, the metabolism of the myeloblasts is obscured by the respiration and splitting metabolism of the mature granulocytes. The problem resolves itself into the obtaining of uninjured immature granulocytes without an admixture of mature white blood cells and of erythrocytes. The actual separation of the mature and immature white blood cells in amounts sufficient for metabolism determination is impossible. Calculation of the metabolism figures of the immature cells by merely subtracting metabolism figures of the mature cells according to their proportion in the differential cell count, is likewise impossible because of the great variability of the metabolism figures of the mature cells, as shown in Table I. Moreover the differential cell count is irrelevant since only the differential cell weight here is essential. The separation of red and white blood cells seems easy at first glance, but it is just this apparent ease which is the main source of error in experiments with these sensitive cells which above all must remain uninjured in order to survive in a normal condition. Even simple procedures such as centrifuging or sedimenting the blood by allowing it to be stationary for any length of time, or exposure to low temperatures are sufficient to injure the cells considerably so that it may well occur that it is not the difference in metabolism of the various types of leukocytes that is measured but only the difference in their resistance to various injuries.

Since, therefore, a material of uninjured immature granulocytes without admixture of mature granulocytes and red blood cells obviously cannot be obtained by experimental means investigations of the metabolism of leukemic immature cells would be impossible but for the rare opportunity of meeting with a case of leukemia in which the leukocyte count is high in comparison to the red blood cell count, and the proportion of myelo-

blastic cells to mature white cells is so great that the metabolism of both red and mature white blood cells does not enter into consideration. Such cases occur with extreme rarity and even among cases of myeloblastic leukemia cited in the literature with a white blood cell count above 100 000, a differential count of more than 70 to 80 per cent of myeloblasts is not often mentioned. The presence of only 20 to 30 per cent of mature white cells, however, may suffice to prevent the exact determination of the metabolism of the myeloblasts themselves. In nine years of studying the metabolism of blood cells, I have encountered only a single case presenting the conditions required. This patient had a white cell count of 180 000 with about 95 per cent of immature myeloblastic cells.

In preparing the blood specimen for the metabolism experiment great care was taken to avoid all injury to the cells. Blood was taken from the cubital vein in heparin (1 mgm. per 2 c.cm of blood) and very gently shaken with glass beads in a cylinder for 3 minutes. The blood should not be taken in oxalate or citrate solution, it should not be diluted and it should be saturated at body temperature with 5 per cent carbon dioxide in air as soon as possible. When the number of leukocytes per c.cm of blood is very great it may happen that within a short time after the removal of the blood from the body so much bicarbonate and sugar has been used up by aerobic glycolysis and respiration, that the aerobic glycolysis to be measured is reduced to a fraction of its optimal rate or may even disappear. A purely oxidative metabolism may thus be simulated which is, however only due to low concentrations of bicarbonate or glucose or to a more acid pH (33). In such instances the sugar and bicarbonate content of the blood as well as its anaerobic glycolysis must be determined in a preliminary experiment, and if necessary enough glucose and sodium bicarbonate added to make sure that during the whole time of the experiment the metabolism is measured under physiological conditions i.e. sugar concentration 1.2 grams per liter, bicarbonate concentration 560 c.mm per c.cm., pH 7.4.

The proportion of white and red blood cells was determined by direct hematocrit reading and checked by comparison with the

and number of red blood cells, assuming that a concentration of five million red blood cells of normal size per c mm corresponds to a cell volume of 45 per cent. The dry weight of the total cell volume was determined according to Peschel (1).

The metabolism determinations were done manometrically. The method, frequently applied (18, 19, 20, 27), of determining aerobic lactic acid formation by measuring the sugar consumption is inadequate, since the measured decrease of the sugar concentration does not indicate how much of the sugar has been used in respiration or in the formation of glycogen (30), and how much has been actually split into lactic acid.

The blood was pipetted into three rectangular manometer vessels, each of about 18 cc. capacity, and into one conical vessel with a large insert well. The side bulbs of all four vessels contained 6 and 3×0.03 c cm $M/17$ lactic acid, previously weighed in. The main space of Vessel I contained 6 c cm of blood, that of Vessels II and III 3 c cm of blood, that of Vessel IV 0.2 c cm of $M/3$ sodium bicarbonate, and the insert well of Vessel IV 2 c cm of blood. The vessels were saturated, while being shaken in the thermostat at 38°C , with gas mixtures prepared over mercury in a gasometer of 2-liter capacity. Vessels I, II, and IV with 5 per cent $\text{CO}_2/95$ per cent air, Vessel III with 5 per cent $\text{CO}_2/45$ per cent $\text{CO}/50$ per cent N_2 . The shaking speed employed was 180 oscillations per minute. Readings were made every five minutes without stopping the manometers. The figures remained constant for over one hour. After this time, or in other experiments after 20 minutes, the lactic acid was tipped in from the side bulbs. The retention of carbon dioxide and of lactic acid

by the blood cells, as well as the metabolism figures for oxygen consumption, carbon dioxide formation, and lactic acid formation were calculated according to Warburg (34).

Table II shows the blood metabolism of the patient with myeloblastic leukemia and of a normal woman of the same age. Calculated per mgm of dry weight of cells, the anaerobic lactic acid formation of the myeloblastic blood is 7.6 times greater, the respiration 47 times greater than that of normal blood. The absolute metabolism figures of respiration and anaerobic glycolysis are not important as compared with the finding that no lactic acid is formed by the myeloblastic blood in air, and that the aerobic glycolysis-respiration ratio, in the blood of the patient with myeloblastic leukemia, is 0 to 235 against 45 to 5 in normal blood.

From a comparison of the metabolism figures of myeloblastic blood to those of normal blood one can obtain the metabolism figures of the myeloblasts themselves, as shown in Table III.

The absolute value of the rate of respiration is of the same magnitude as that of immature leukemic lymphocytes and of human erythroblasts, 154 times greater than that of mature erythrocytes, but only one-third of that of human exudate leukocytes. The respiratory quotient of myeloblasts in air is 0.75. The rate of anaerobic glycolysis of leukemic granulocytes and leukemic lymphocytes is nearly the same. 11.5 and 11.1 c mm of lactic acid is formed in one hour by 1 mgm (dry weight) of cells, against 58 c mm formed by human exudate leukocytes, 51 c mm formed by human nucleated red blood cells, and 0.5 c mm formed by mature erythrocytes (Table IV). An amount of lactic acid equal to the cell

TABLE II

Metabolism of myeloblastic and of normal blood (calculated per 100 mgm of total blood cell dry weight and per 1 hour)

	I Respiration (c.mm oxygen consumed in air)	II Aerobic glycolysis (c.mm * lactic acid formed in air)	III Anaerobic gly- colysis (c.mm * lactic acid formed in absence of oxygen)	IV Inhibition of lactic acid formation by air	V Ratio of aerobic glycolysis to respiration $\frac{II}{I}$
Blood of patient with myeloblastic leukemia	235	0	380	per cent 100	0
Blood of normal woman	5	45	50	10	9

* 1 c.mm = 0.004 mgm of lactic acid

TABLE III
Calculation of metabolism figures for 1 mgm. (dry weight) of myeloblasts

	I c.mm oxygen consumed in air in 1 hour	II c.mm * lactic acid formed in air in 1 hour	III c.mm. lactic acid formed in absence of oxygen in 1 hour	IV Inhibition of lactic acid formation by air	V Aerobic lactic acid formation to respiration $\frac{II}{I}$
100 mgm of blood cells of patient with myeloblastic leukemia (30 per cent white blood cells 70 per cent red blood cells)	235.0	0	380	per cent 100	0
Minus 70 mgm of blood cells of normal person (70 per cent of the cells of the myeloblastic blood)	3.5	31.5	35	10	9
30 mgm of myeloblasts (30 per cent of the cells of myeloblastic blood)	231.5	0	345	100	0
1 mgm of myeloblasts	7.7	0	11.5	100	0

* 1 c.mm = 0.004 mgm of lactic acid

TABLE IV
Metabolism of human blood cells (calculated per 1 mgm of dry weight of cells per hour)

Cell species	I Q_{O_2} Respiration (c.mm oxygen consumed in air)	II Q_{O_2} Aerobic glycolysis (c.mm lactic acid formed in air)	III $\frac{CO_2}{O_2}$ Anaerobic gly- colysis (c.mm. lactic acid formed in absence of oxygen)	IV Inhibition of lactic acid formation by air	V $\frac{II}{I}$ Aerobic lactic acid formation to respiration
Erythroblasts (35)	12.80	24.30	50.6	per cent 52	1.92
Erythrocytes	0.05	0.45	0.5	10	9.00
Exudate leukocytes (22-35)	22.80	16.80	57.8	71	0.74
Leukemic lymphocytes (1)	5.80	0	11.1	100	0
Myeloblasts	7.70	0	11.5	100	0

dry weight is formed under anaerobic conditions by myeloblasts in 22 hours, by erythroblasts in 5 hours by erythrocytes in 500 hours by exudate leukocytes in a little over 4 hours. Under aerobic conditions the same amount of lactic acid is formed by erythroblasts in 10 hours by exudate leukocytes in 15 hours by erythrocytes in 550 hours. Lymphoblasts and myeloblasts form no lactic acid aerobically.

The metabolic features of the maturity process of red blood cells are well seen in the metabolism figures of human erythroblasts and erythrocytes (35). The rate of respiration as well as that of lactic acid fermentation is considerably lower in the older than in the younger cells. But since the rate of respiration has decreased much more than the rate of fermentation (250 times against 50

times) a disturbance of the aerobic glycolysis respiration ratio results. This ratio in mature erythrocytes is 9 in erythroblasts 2. Table V contrasts the white blood cell metabolism in the case of pure myeloblastosis with that in cases of myelogenous leukemia with a smaller proportion of immature cells. In Case I the proportion of the more mature cells (segmented juvenile and myelocytic granulocytes, and lymphocytes) to the myeloblasts is 98:2 in Case II 50:50 in Case III 5:95. The aerobic glycolysis respiration ratio is in Case I 7.2 in Case II 1 in Case III 0.

As demonstrated for red blood cells in white blood cells also the increase of the aerobic glycolysis-respiration ratio is evidence only of the increasing age or the maturity of the cells. The figures of Table V, especially of Column V,

TABLE V

Blood metabolism of three cases of myelogenous leukemia with different proportions of myeloblasts to more mature granulocytes

	Total white blood cell number per c mm	Lymphocytes	Segmented stab and juvenile forms	Myelocytes	Myeloblasts	Proportion of more mature granulocytes to myeloblasts	I Respiration Q_{O_2}	II Aerobic glycolysis Q_{O_2}	III Anaerobic glycolysis Q_{CO_2}	IV Inhibition of glycolysis by air per cent	V Ratio of aerobic glycolysis to respiration $\frac{II}{I}$
I	186,000	2	67	29	2	98 2	2 8	20 2	24 6	18	7 2
II	52,000	3	18	29	50	50 50	2 4	2 4	5 3	55	1 0
III	179,000	2	1	2	95	5 95	7 7	0	11 5	100	0

quantitatively why former investigators (18, 20, 27) ascribed the properties of cancer-cell metabolism to leukemic myelogenous cells. All the instances of myelogenous leukemia appearing in the literature, where metabolism determinations were made, belong to the same group as Cases I and II of our Table V, *i e*, the leukocytes examined were not young immature leukemic granulocytes but mixtures of "old" mature and "young" immature cells in which these old and dying cells predominated to so great an extent that the characteristic metabolism of the myeloblasts as such was entirely obscured.

The question, therefore, of the nature of the leukemic myeloblastic cell is definitely answered, it seems to me, by the results on the energy supplying reactions of the myeloblastic cells. The metabolism of myeloblasts is purely oxidative, there is no aerobic splitting of sugar into lactic acid which is invariably found in benign and, to a greater degree, in malignant tumor cells, in injured cells, mature leukocytes, and erythrocytes. Even the aerobic glycolysis of the erythrocytes, which constitute 70 per cent of the cell volume of the blood examined is, in the manometrical experiment, completely overshadowed by the high myeloblastic respiration. Myeloblasts, contrary to the mature blood and exudate leukocytes of healthy and leukemic people, possess the characteristic metabolism of uninjured normal young cells. From the standpoint of cellular physiology one may say definitely that the metabolism of myeloblasts differs fundamentally from that of malignant tumor cells.

In previous experiments (22) we examined the chemical composition of tissue fluids in normal and inflamed areas and found a disturbed equilibrium

of oxygen, carbon dioxide, sugar, lactate, and bicarbonate as a result of inflammation. The products of the metabolism of exudate leukocytes which accumulated in the closed manometer vessel, also accumulated in very high amounts in the inflamed area, and were not carried off by the blood or lymph flow. After an inflammation period of three days, the sugar concentration of sterile blisters of the skin, for instance, was zero against 100 mgm per cent in normal tissue fluid, the sodium lactate concentration was above 125 mgm per cent against 10 mgm per cent, the bicarbonate had decreased to half its amount and the pH had dropped to values as low as 6.2. We stated then, as our opinion, that these chemical changes, due for the most part to the aerobic glycolysis of the leukocytes, play an important role in the fight of the body against bacteria, since under these unfavorable conditions of the nutrient medium bacteria are severely damaged, or even starved to death. This opinion was supported by von Bergmann (26) and Lohmann (33, 36).

Since, in contrast to the mature white blood cells, the myeloblastic cells lack completely aerobic glycolysis, they are unable to produce any of the above mentioned chemical effects. The function of the mature leukocytes, therefore, can not be taken over by the more immature leukemic cells, and it is obvious why an increase of immature leukocytes with a decrease of mature forms leads to serious danger whenever pathogenic bacteria attack the body. The detrimental factor is not the presence of myeloblasts, or lymphoblasts, but the absence of mature white cells capable of forming lactic acid under aerobic conditions.

For the vague concept of "weakened resistance" of patients with myeloblastic or lymphoblastic

leukemia toward bacterial infection, we may substitute a definite quantitatively measurable chemical fact, namely, that the purely oxidative metabolism and total lack of aerobic glycolysis of the myeloblasts and lymphoblasts renders them unable to carry out the bactericidal "reactions of inflammation."

CONCLUSIONS

1 Whether leukemic cells are malignant or benign tumor cells or normal young tissue cells, can not be decided by morphological investigation. The question can be answered definitely by studies of the metabolic reactions of leukemic blood cells.

2 The metabolism of the blood cells from 15 patients with lymphatic leukemia and from 40 patients with myelogenous leukemia was determined manometrically with the Warburg method. Myeloblasts as well as lymphoblasts—in contrast to the more mature forms of leukocytes—have a purely oxidative metabolism and do not form lactic acid under aerobic conditions. The aerobic glycolysis respiration ratio in the blood of a patient with myeloblastic leukemia (180 000 white cells per c.mm. 95 per cent myeloblasts) was 0 to 235, against 45 to 5 in normal blood. The anaerobic glycolysis of the myeloblastic blood was 7.6 times the respiration 47 times greater than that of normal blood. The respiratory quotient of myeloblasts, measured in air, was 0.75. Aerobic glycolysis which occurs (independent of the rate of respiration) in more mature leukocytes was found to be a symptom of their ageing and dying off. Myeloblasts as well as lymphoblasts exhibit the characteristic metabolism of uninjured normal young cells and not that of cancer cells.

3 The weakened resistance of patients with myeloblastic or lymphoblastic leukemia toward bacterial infection is explained by the absence of aerobic glycolysis in immature leukemic blood cells.

BIBLIOGRAPHY

- 1 Peschel E. Stoffwechsel leukämischer Lymphocyten. *Klin. Wehnschr.*, 1930 2, 1061
- 2 Virchow R., Die krankhaften Geschwulste. Bd. II, Hirschwald, Berlin, 1864 p. 568.
- 3 Banti, G. Die Leukämien. *Centralbl. f. allg. Path. u. path. Anat.*, 1904 15 1.
- 4 Ribbert, H., Menschliche Zellen als Parasiten. *Deutsche med. Wehnschr.*, 1907 33 329
- 5 MacCallum, W. G., A Textbook of Pathology W. B. Saunders Co., Philadelphia, 1936 6th ed., p. 875
- 6 Boyd, W., The Pathology of Internal Diseases. Lea and Febiger Philadelphia, 1935 2d ed. p. 618
- 7 Ewing J., Neoplastic Diseases. W. B. Saunders Co., Philadelphia, 1928 3d ed. p. 397
- 8 Richter M. N., and MacDowell E. C., Experiments with mammalian leukemia. *Physiol. Rev.*, 1935, 15 509
- 9 Sternberg C., Blutkrankheiten, Leukämien In Henke-Lubarsch, Handb. d. spez. Path. Anat. u. Hist., 1926 1 56
- 10 Naegeli, O., Blutkrankheiten und Blutdiagnostik. Springer Berlin, 1931 5th ed.
- 11 Warburg, O., Stoffwechsel der Tumoren. Springer Berlin, 1926.
- 12 Grafe, E., Die Steigerung des Stoffwechsels bei chronischen Leukämie und ihre Ursachen. *Deutsches Arch. f. klin. Med.*, 1911 102, 406
- 13 Levene, P. A. and Meyer G. M., On the action of leukocytes on glucose. *J. Biol. Chem.*, 1912, 12, 265
- 14 Burger, M., Untersuchungen über Hämoglykolyse. *Ztschr. f. d. ges. exper. Med.*, 1923 31 1
- 15 Bakker A., Einige Uebereinstimmungen im Stoffwechsel der Carcinomzellen und Exsudatleukocyten. *Klin. Wehnschr.*, 1927 6, 252.
- 16 Fleischmann, W., and Kubowitz, F., Ueber den Stoffwechsel der Leukocyten. *Biochem. Ztschr.*, 1927, 181 395
- 17 Fujita, A., Ueber den Stoffwechsel der Körperzellen. *Biochem. Ztschr.*, 1928 197, 175
- 18 Barron, E. S. G., and Harrop, G. A. Jr., Studies on blood cell metabolism. Metabolism of leukocytes. *J. Biol. Chem.*, 1929 84 89
- 19 Jackson, H., Parker F., and Glover E. C., Studies of diseases of the lymphoid and myeloid tissues. I. *J. Exper. Med.*, 1930 52, 547
- 20 Glover E. C., Daland, G. A. and Schmitz, H. L., The metabolism of normal and leukemic leukocytes. *Arch. Int. Med.*, 1930 46, 46.
- 21 Schlossmann, H., Ueber den Stoffwechsel von Lymphocyten. *Biochem. Ztschr.*, 1930 219 463.
- 22 Kempner W. and Peschel, E., Stoffwechsel der Entzündung. *Ztschr. f. klin. Med.*, 1930 114 439
- 23 Loebel R. O., Shorr E., and Richardson H. B., The influence of adverse conditions upon the respiratory metabolism and growth of human tubercle bacilli. *J. Bact.*, 1933 26 167
- 24 Fleischmann W., Pathologische Physiologie des Stoffwechsels weisser Blutzellen. *Wien. med. Wehnschr.* 1933 83, 215
- 25 Fleischmann, W., Der Stoffwechsel des geschädigten Gewebes. *Naturwissenschaften*, 1936 24 15
- 26 von Bergmann, G., Funktionelle Pathologie. Springer Berlin, 1932, p. 170
- 27 Soffer L. J. and Wintrobe, M. M. The metabolism of leukocytes from normal and leukemic blood. *J. Clin. Invest.*, 1932, 11 661.

- 28 Horsters, H, Vergleichende Versuche ueber die Glykolyse durch myeloische und lymphatische Leukocytenformen des menschlichen Blutes Ztschr f d. ges exper Med, 1936, 97, 479
- 29 Bossa, G, Sul metabolisma dei leucociti leucemici Haematologica, 1937, 18, 673
- 30 Willstätter, R., and Rohdewald, M., Ueber Aufbau und Abbau des Glykogens durch Leukocyten (Zehnte Abhandlung ueber Enzyme der Leukocyten) Ztschr f physiol. Chem., 1937, 247, 115
- 31 Victor, J, and Potter, J S, Studies in mouse leukemia. XI Metabolic effects of host constitution J Exper Med, 1934, 60, 547
 Studies in mouse leukemia Pre-leukemic changes in lymphoid metabolism. Brit J Exper Path, 1935, 16, 234
 Studies in mouse leukemia Metabolic observations in spontaneous lymphatic leukemia. Brit. J Exper Path, 1935, 16, 253
- 32 Victor, J, and Wintersteiner, M B, Studies in mouse leukemia. X Metabolic differences between transmission lines of mouse lymphatic leukemia Am J Cancer, 1934, 22, 561
- 33 Lohmann, R., Zellstoffwechsel und Entzündung Klin Wehnschr, 1938, 17, 427
 Biologie der Entzündung Ztschr f klin Med, 1938, 135, 316
- 34 Warburg, O, Kubowitz, F, and Christian, W, Ueber die Wirkungen von Phenylhydrazin und Phenylhydroxylamin auf den Stoffwechsel von roten Blutzellen Biochem Ztschr, 1931, 242, 170
- 35 Kempner, W, Metabolism of human erythroblasts J Clin Invest, 1936, 15, 679
- 36 Lohmann, R., Krebsstoffwechsel und Entzündung Klin. Wehnschr, 1931, 10, 1799
 Manometrische Untersuchungen ueber Stoffwechsel und Wachstum von Bakterien unter dem Einfluss von ultraviolettem Licht und unter den Bedingungen der Entzündung Klin Wehnschr, 1934, 13, 1112

THE ANTIKETOGENIC ACTIVITY OF SUCCINIC ACID

By EATON M. MACKAY, JAMES W. SHERRILL, AND RICHARD H. BARNES

(From The Scripps Metabolic Clinic, La Jolla)

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Korányi and Szent Gyorgyi have reported (1, 2) that relatively small doses of succinic acid would reduce or abolish the ketosis in diabetes. We have been unable to demonstrate this action of succinic acid. In Table I are presented three typical examples of the result of administering succinic acid to diabetic patients. All of these patients had been on their régime for some time and their diets were constant. They were given the free acid. The total ketone bodies in the

TABLE I

The influence of succinic acid on ketosis in diabetes upon a fixed régime
Urine excretion per day

Day	Total ketone bodies	Nitrogen	Glucose	Succinic acid per day
	GRAMS	GRAMS	GRAMS	GRAMS

Experiment 1*

1	3.21		10.2	
2	4.50		13.4	
3	3.96		7.0	
4	4.22		7.9	
5	5.28		9.3	10
6	4.12		12.5	10
7	5.30		22.3	10
8	4.20		18.4	10
9	5.40		21.3	10
10	2.10		3.2	
11	1.40		5.4	
12	0.09		1.1	

Experiment 2†

1	1.02		3.2	
2	0.88		4.4	
3	0.74		4.8	5
4	1.10		4.6	5
5	0.68		3.2	5
6	1.30		5.8	5
7	2.80		20.2	30
8	2.10		4.3	
9	1.20		2.2	

* Experiment 1. Female, 48 years of age, weighing 54.5 kgm. and receiving 24 units of insulin each day. The daily diet was composed of protein 85 grams, fat 143 grams, and carbohydrate 120 grams. The dose of insulin was increased to 34 units on the tenth day.

† Experiment 2. Male, 26 years of age, weighing 75.6 kgm. and receiving 30 units of insulin each day. The daily diet was composed of protein 120 grams, fat 170 grams, and carbohydrate 200 grams. On the eighth and ninth days 42 units of insulin were given.

TABLE I—Continued

Day	Total ketone bodies	Nitrogen	Glucose	Succinic acid per day
	GRAMS	GRAMS	GRAMS	GRAMS
Experiment 3‡				
1	2.81	8.9	8.2	
2	3.08	7.1	5.7	
3	0.68	6.5	8.6	
4	1.34	10.9	3.0	
5	2.56	13.5	33.9	50
6	2.34	9.7	23.6	15
7	1.71	8.5	5.6	
8	2.53	8.4	11.7	
9	2.07	9.9	13.0	

‡ Experiment 3. Female, 17 years of age, weighing 41.3 kgm. and receiving 28 units of insulin per day. The daily diet was composed of protein 55 grams, fat 140 grams, and carbohydrate 150 grams.

urine were determined by Van Slyke's method (3), glucose according to Benedict (4), and total nitrogen by the macro Kjeldahl (5). Small doses (Table I, Experiment 1) of succinic acid had no demonstrable effect upon the ketosis as measured by the ketonuria. This confirms the observations of several English investigators (6, 7). Larger doses of succinic acid had quite the opposite effect to the action claimed by Korányi and Szent Györgyi. The ketonuria was actually increased (Table I, Experiments 2 and 3). In these cases the compound behaved in the same manner as might have been expected of glucose. This is hardly surprising for succinic acid is converted to glucose in the phloridzinized dog (8, 9).

In the fasting normal human being the glucose effect of succinic acid is even more obvious (Table II). Contrary to its behavior in the diabetic, succinic acid is nitrogen sparing and has marked antiketogenic activity such as might be expected from an equivalent amount of glucose in a subject of this kind. The antiketogenic activity of succinic acid in the normal fasting organism may also be demonstrated in fasting rats in which a ketonuria has been induced by feeding the sodium salt of β -hydroxybutyric acid (Table III). If

TABLE II

*The influence of succinic acid upon the ketosis of a fasting normal subject**

Day	1	2	3	4	5	6
Total urine nitrogen, grams	5.13	7.00	6.44	5.12	4.03	7.91
Total ketone bodies, grams	2.17	5.87	5.96	2.23	0.45	6.70
Succinic acid fed, grams				50	50	

* This individual was a female who was normal in every respect except for being slightly (5 to 10 kgm) overweight. She was 38 years old and weighed 71 kgm. A general diet preceded the fast. While fasting, three cups of coffee without additions were allowed each day. The succinic acid was fed in dilute solution.

TABLE III

*Antiketogenic action of succinic acid in fasting rats**

Group	Body weight	Body surface	Total nitrogen excreted per rat per day				Total ketone bodies excreted per rat per day			
			Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
A	grams	sq. dm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
B	261	4.63	47	56	43	51	50	72	72	74
	256	4.56	41	45	35	39	15	13	13	11

* There were 10 male rats in each group which were fasted for a day before the experiment and throughout the experimental period. During the latter they were all given 1 cc. per sq. dm. of body surface of a 6.3 per cent solution of racemic sodium β -hydroxybutyrate twice each day. The solution given to Group B contained 5.9 per cent of succinic acid in addition.

sufficient of the succinic acid is administered the ketonuria will completely disappear as may be seen in another experiment.

It is reasonable to assume that succinic acid is antiketogenic in the normal organism because it is converted to glucose. The relative activity of these compounds in this regard should throw some light upon this point. An excellent ketosis may be produced in rats if they are fasted after a period upon a diet low in choline and protein which results in a fatty liver (10, 11, 12). When a high fluid intake is provided so as to insure large urine volumes the ketonuria becomes a good measure of the degree of ketosis. The ketosis increases to a maximum on about the third day of fasting and then tapers off. In comparing the antiketogenic action of the two compounds two millimols of succinic acid were fed for comparison with each millimol of glucose. This is the theoretical relation between these substances if three carbon atoms of succinic acid go to glucose (8), and under optimal conditions one millimol of

glucose may be obtained from each two millimols of succinic acid fed to the phloridzinized dog (8, 9). Our results in Table IV show that succinic acid has essentially the same antiketogenic activity as an equivalent amount of glucose.

Substances which exhibit antiketogenic activity are generally, if not always, good glycogen formers (13, 14). Succinic acid is no exception. The results in Table V demonstrate that this compound is practically as good a glycogen former as is glucose.

DISCUSSION

We are unable to explain why we and others (6, 7) have been unable to corroborate in any degree the findings of Koranyi and Szent-Gyorgyi concerning the desirable therapeutic effect of succinic acid in human diabetes in that the ketosis may be reduced or controlled by this compound. It is probable that their patients had not been adequately controlled before the study and were exhibiting the benefit of other therapeutic measures which had been showing their effects slowly when the succinic acid happened to be given. There is also, of course, the very remote possibility that if diabetics differ in the cause of their disturbed metabolism one type of case might be benefited by succinic acid, and it happened that the four patients treated by these authors were of this type.

Although not absolute proof, the fact that the antiketogenic activity of succinic acid is proportional to the glucose it may form is strong evidence that the antiketogenic action of succinic acid in the intact organism is simply a result of its conversion to glucose and is not due to any catalytic action on metabolism such as Szent-Gyorgyi and coworkers (15) and others (16) believe possible.

Our conclusion that in the normal organism succinic acid is as good an antiketogenic agent as an equivalent quantity of glucose is opposed to the conclusions of Deuel *et al.* (17) regarding the activity of this substance. A perusal of their data shows why they failed to obtain the same results. They fed sodium succinate. The succinic acid was oxidized leaving a substantial amount of alkali. Alkali is very ketogenic (18, 19) so that while their glucose-fed group had only the formation of ketones by the fasting fatty

TABLE IV
The relative antiketogenic activity of succinic acid and glucose in fasting rats

Group	Sex	Body weight	Body surface*	Excretion per sq. dm. of body surface per day								Dose per sq. dm. of body surface per day		
				Total ketone bodies				Total nitrogen				NaHCO ₃	Glucose	Succinic acid
				Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4			
		grams	sq. cm	mgm	mgm	mgm	mgm	mgm	mgm	mgm	mgm.	mgm	mgm.	mgm.
<i>Experiment 1†</i>														
A	♂	201	390	1	4	54	13					2.0		
B	♀	188	373	2	41	77	29					2.0		
C	♀	207	397	0	2	21	14					2.0	0.25	
D	♀	161	336	1	10	52	27					2.0	0.25	
E	♀	211	403	0	0	0	0					2.0	0.50	
F	♀	161	336	1	1	7	0					2.0	0.50	
G	♀	211	403	1	5	31	10					2.0		0.5
H	♀	188	373	2	9	37	34					2.0		0.5
I	♀	222	416	0	0	0	0					2.0		1.0
J	♀	161	336	1	1	3	0					2.0		1.0
<i>Experiment 2‡</i>														
A	♀	167	344	46	53	36	61	22	17	16	17	2.0		
B	♀	165	340	27	19	15	16	18	14	13	18	2.0	0.33	
C	♀	165	340	5	1	0	0	15	13	14	16	2.0	0.67	
D	♀	167	344	33	26	19	22	15	15	15	15	2.0		0.5
E	♀	166	342	9	8	1	0	16	14	14	15	2.0		1.0
<i>Experiment 3§</i>														
A	♂	161	336	19	20	28	15							
B	♀	165	340	46	30	36	5							
C	♀	171	350	0	2	0	0						0.25	
D	♀	168	345	0	6	9	4						0.25	
E	♀	161	336	0	0	0	0						0.75	
F	♀	159	333	0	0	0	0						0.75	
G	♀	186	370	0	2	2	0							0.5
H	♀	157	330	0	8	6	1							0.5
I	♀	183	366	0	0	0	0							1.5
J	♀	165	340	0	0	0	0							1.5
<i>Experiment 4 </i>														
A	♂	151	322	4	10	8	6	27	24	23	19	2.5		
B	♀	138	304	3	15	10	6	31	29	27	22	2.5		
C	♀	157	330	0	0	1	0	22	27	26	18	2.5	0.25	
D	♀	144	310	0	1	0	0	23	24	26	16	2.5	0.25	
E	♂	150	321	0	2	0	0	33	29	26	21	2.5		0.5
F	♀	137	302	1	0	0	0	29	27	24	22	2.5		0.5

* In all of these experiments "Body surface" was calculated from the formula (20) that we have generally used. The methods of urine collection and administering the various solutions have already been described (22, 23).

† Rats which had been on the low protein fatty liver producing diet (12) for 17 days were used. Urine collections were made and the various solutions administered beginning on the first day of fasting. There were 6 rats in each group the averages for which are given here. The solutions were administered in two doses each day: the succinic acid in 2.95 and 5.90 per cent, the glucose in 2.25 and 4.50 per cent, and the NaHCO₃ in 8.4 per cent solution.

‡ The rats had been on the special diet for 15 days. Collections were made on and after the second day of fasting. Due to an error in calculation (the incorrect assumption being made that all four carbon atoms of succinic acid form glucose) the glucose fed rats received more glucose than the equivalent of the succinic acid fed, and the antiketogenic action was consequently greater. The concentration in the solutions of succinic acid was 2.95 and 5.90 per cent of glucose 3.0 and 6.0 per cent, and of NaHCO₃ 8.4 per cent. The results are averages for 5 rats in each group.

§ In this experiment there were 5 rats in each group and they had been on the fatty liver producing diet for 17 days. Urine collections were commenced on the first day of fasting. The succinic acid solutions were not neutralized, nor was NaHCO₃ fed to any of the other groups. All of the rats were given 1 cc. per sq. dm. of body surface of 1 per cent NaCl twice each day and other substances administered were incorporated in this glucose, in 2.25 and 7.75 per cent and succinic acid in 2.95 and 5.94 per cent solution.

|| These rats were fasted directly from the stock diet. Collections were commenced on the second day of fasting. The ketonuria is due solely to the alkalosis produced by the NaHCO₃. This was fed in 10.5 per cent, the glucose in 2.25 per cent and the succinic acid in 2.95 per cent solutions. The results are averages for 6 rats in each group.

TABLE V
Glycogenic activity of succinic acid and glucose in the albino rat*

Group	Body weight	Body surface	Liver weight	Liver glycogen				Amount fed per sq cm of body surface		
				Minimum	Maximum	Average	Average	NaHCO ₃	Glucose	Succinic acid
	grams	sq. dm.	grams	per cent	per cent	per cent	mgm. per sq cm of body surface	mm	mm	mm
A	150	3.20	4.12	0.2	0.8	0.6	8	5.0		
B	149	3.18	4.92	7.1	15.4	9.6	148	5.0	1.25	
C	150	3.21	4.82	6.3	12.9	8.8	138	5.0		2.50

* Each group was composed of 12 male rats. They were fasted for 24 hours and then given by stomach tube every 4 hours 1 cc. per sq dm. of body surface of the various solutions over a period of 20 hours, when they were anesthetized with nembutal and the liver glycogen determined (21). All of the solutions contained 8.4 per cent NaHCO₃. In Group B there was 4.5 per cent glucose and in Group C 5.9 per cent succinic acid in addition.

liver rats to overcome, the group fed sodium succinate had in addition the ketogenic influence of the alkali to overcome. Experiment 4 in Table IV shows the effect on ketosis of alkali in the form of a dose of sodium bicarbonate comparable to the alkali involved in these experiments. When fasting from the stock diet without alkali administration there is no measurable ketonuria. Whenever we fed sodium succinate we naturally added an equivalent amount of sodium bicarbonate to the control and glucose-fed groups so that the observations in a given experiment would be comparable.

SUMMARY

Succinic acid has no antiketogenic activity in the human diabetic. In a normal fasting person it is as antiketogenic as an equivalent amount of glucose, to which succinic acid is converted in the phloridzinized and probably also in the normal organism.

The ketosis of fasting rats which previously had been receiving a fatty liver producing diet is reduced in the same degree by glucose as by an equivalent amount of succinic acid. These compounds are also almost equally good glycogen formers.

BIBLIOGRAPHY

- Koranyi, A., and v. Szent-Györgyi, A., Curing acidosis of diabetics by means of succinic acid treatment. *Orvos i hetil.*, 1937, 81, 615. Cited from *Chem. Abstr.*, 1937, 31, 6335.
- Koranyi, Andreas and v. Szent-Györgyi, Albert, Ueber die Bernsteinsäurebehandlung diabetischer Azidose. *Deutsche med. Wchnschr.*, 1937, 63, 1029.
- Van Slyke, D. D., Studies in acidosis. VII. The determination of β -hydroxybutyric acid, acetoacetic acid, and acetone in urine. *J. Biol. Chem.*, 1917, 32, 455.
- Benedict, S. R., The detection and estimation of glucose in urine. *J. A. M. A.*, 1911, 57, 1193.
- Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Vol. II, Methods. Williams and Wilkins Co., Baltimore, 1932, p. 534.
- Lawrence, R. D., McCance, R. A., and Archer, N., Clinical memoranda. Succinic acid treatment of diabetic ketosis. *Brit. M. J.*, 1937, 2, 214.
- Dunlop, D. M., and Arnott, W. M., Effect of succinic acid on diabetic ketosis. *Lancet*, 1937, 2, 738.
- Ringer, A. I., Frankel, E. M., and Jonas, L., The chemistry of gluconeogenesis. IV. The fate of succinic, malic, and malonic acids in the diabetic organism, with consideration of the intermediary metabolism of aspartic and glutamic acids, proline, lysine, arginine, and ornithine. *J. Biol. Chem.*, 1913, 14, 539.
- MacKay, E. M., and Barnes, R. H., Conversion of succinic acid to glucose in the phloridzinized dog. *Proc. Soc. Exper. Biol. and Med.*, 1938, 38, 417.
- Best, C. H., and Channon, H. J., The action of choline and other substances in the prevention and cure of fatty livers. *Biochem. J.*, 1935, 29, 2651.
- Channon, H. J., and Wilkinson, H., Protein and the dietary production of fatty livers. *Biochem. J.*, 1935, 29, 350.
- MacKay, E. M., The influence of a pancreas extract ("fat metabolizing hormone") upon fat deposition in the liver on a low protein diet. *Am. J. Physiol.*, 1937, 119, 783.
- Shapiro, I., Studies on ketosis. V. The comparative glycogenic and ketolytic action of glucose and some carbohydrate intermediates. *J. Biol. Chem.*, 1935, 108, 373.
- Mirsky, I. A., Heiman, J. D., and Broh-Kahn, R. H., The antiketogenic action of glucose in the absence of insulin. *Am. J. Physiol.*, 1937, 118, 290.

- 15 Annau, E. Banga, I. Blazsó A., Bruckner, V., Lakd, K., Straub F B and Szent Gyorgyi, A., Ueber die Bedeutung der Fumarsäure für die tierische Gewebsatmung Ztschr. f. physiol. Chem., 1936 244 105
- 16 Krebs H. A. The intermediate metabolism of carbohydrates. *Lancet*, 1937 2 736.
- 17 Deuel H. J., Jr., Murray S and Hallman, L., A comparison of the ketolytic effect of succinic acid with glucose. *Proc. Soc. Exper Biol. and Med.*, 1937 37 413
- 18 Beumer H., and Soecknick, A., Ueber organische Acidose bei anorganischer Acidose und Alkalose. *Ztschr. f. Kinderh.*, 1924 37, 236.
- 19 Porges O., and Lipschütz, H., Ueber Azetonurie und Alkalose. *Arch. f. exper Path. u. Pharmacol.*, 1923 97 379
- 20 Carman, G. G., and Mitchell, H. H., Estimation of the surface area of the white rat. *Am. J. Physiol.*, 1926 76, 380
- 21 Good, C. A., Kramer, H. and Somogyi, M., The determination of glycogen. *J. Biol. Chem.*, 1933 100 485.
- 22 MacKay E. M., and Barnes R. H., Influence of adrenalectomy on ketosis of fasting and on the action of the anterior pituitary ketogenic principle. *Am. J. Physiol.*, 1937 118, 184
- 23 MacKay, E. M. and Barnes, R. H., Influence of adrenalectomy upon ketolytic activity *Am. J. Physiol.*, 1938, 122 101

IMMUNOLOGICAL STUDIES IN PATIENTS WITH PNEUMOCOCCUS TYPE III PNEUMONIA TREATED WITH SUL- FANILAMIDE AND SERUM

By MAXWELL FINLAND AND JOHN W. BROWN

(From the Thorndike Memorial Laboratory Second and Fourth Medical Services (Harvard)
Boston City Hospital and the Department of Medicine Harvard Medical
School Boston)

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During the past season (November 1937 to May 1938) we had an opportunity to study the immune reactions of a group of patients with *Pneumococcus* Type III pneumonia before and during treatment with sulfanilamide and type-specific antipneumococcal rabbit serums used separately or in combination. Observations were also made on the effect of these substances *in vitro*, on the bactericidal action of the blood of the patients taken before treatment. In this paper we present the results of these tests and attempt to correlate them with the course of the disease. Clinical results are reported in a separate communication (1).

The irregular and atypical response of animals to immunization with Type III pneumococci has been reported by a number of workers (2). The antibody response of human beings to Type III pneumococcal pneumonia (3) or to immunization with the specific carbohydrate of this type (4) is quite similar to that of other common pneumococcal types (3, 4). The blood of many normal adults and children has bactericidal properties against the Type III pneumococcus (5). Thus bactericidal power is inhibited by the type-specific capsular polysaccharide and is enhanced by specific antiserum (5a, 6). The latter can also neutralize the antibactericidal action of the specific carbohydrate when used in proper concentrations (6).

Robertson and his coworkers (7) studied the pneumococcal-promoting action of the serum of 3 patients during the course of Type III pneumonia. There was some activity on the first or second day in each instance. One patient had a negative blood culture throughout the course of the disease and recovered. A second patient with less pneumococcal promoting activity in the serum had a positive blood culture on the first and second days. Subsequent blood cultures were sterile; there was an increase in the pneumococcal-promoting action of the serum and recovery. The third patient had a high titer of the pneumococcal promoting antibody and a sterile blood culture at 48 hours, but beginning the following day the blood culture became and remained positive and the antibody was no longer demonstrable, the bacteremia increased and the patient died.

Sulfanilamide has been shown to have bacteriostatic and bactericidal action on Type III pneumococci *in vitro* (8). Experimental infections with this organism in rats and mice have been successfully treated with the drug (9). Favorable clinical results in small groups of cases of Type III pneumococcal pneumonia have been reported (10). There were no successfully treated bacteremic cases except 3 recorded by Bullova (11).

MATERIALS AND METHODS

The patients chosen for study each had clinical and x-ray or pathological evidence of pneumonia and Type III pneumococci were identified in one or more specimens of sputum by the Neufeld method (12) and/or isolated after mouse inoculation. Some had typical lobar pneumonia while in others the pulmonary consolidation was atypical in distribution (bronchopneumonia). The non-pneumonic subjects were either laboratory workers or hospital patients free of febrile disease. Blood for culture and for the immunological studies was taken before treatment was begun and at suitable intervals thereafter. For the cultures, 5 to 10 cc. of blood were taken directly into 100 cc. of suitable broth at the bedside and, in addition, 3 cc. were taken in sterile 2.5 per cent citrate solution and agar plates were poured with 1 and 2 cc. of blood as soon as possible after it reached the laboratory. The immunological methods were similar to those used in previous studies (13). In the pneumococcal tests (5a) the sealed tubes containing the defibrinated blood, pneumococci and other added ingredients were observed at 24 and 48 hours and changes in the color of the blood noted. At the end of this period the tube containing the smallest number of pneumococci with which the blood exhibited color change and all those in which the blood remained unaltered were broken open and cultured on the surface of blood agar plates in agar pour plates and frequently also in rabbit's blood broth. Absence of color change was taken to indicate bacteriostasis and failure to obtain pneumococci on culture was considered to indicate bactericidal activity. One of the tubes in each test was inoculated with 0.1 cc. of the original culture and allowed to rotate in the incubator for one hour after which smears were made of its contents and stained with Wright's blood stain. The number of diplococci in 50 consecutive polymorphonuclear leukocytes were counted and the percentage of these cells containing diplococci was noted (6c).

ber of diplococci per polymorphonuclear leukocyte is referred to as the phagocytic index

The Rockefeller Institute strain of Type III pneumococcus in use in this laboratory for several years was employed throughout this study. It was passed through mice almost daily. The cultures used were second or third subcultures of mouse heart's blood in rabbit's blood broth in the lag phase of growth, either after 10 hours' growth with a small inoculum or, when necessary for the pneumococcal test, after 4 or 5 hours' growth with a 50 per cent inoculum. Pour plates yielded 35 to 120 colonies per cc. of the original culture. The virulence was such that either one or both of 2 mice inoculated with 10^{-9} cc. invariably died within 48 hours.

Sulfanilamide determinations in the blood were carried out by the method of Marshall (14). The drug was given to the patients by mouth in divided doses, usually at 4-hour intervals.

The therapeutic immune rabbit serums used were mostly experimental lots of concentrated serums prepared and furnished by the Lederle Laboratories, Inc. The potency of some of these preparations was estimated at 1000 to 5000 units, the unit being defined as 10 times the least amount which protects 50 per cent of mice against 100 fatal doses. No estimations were made on some of the

lots. One preparation of unconcentrated serum was furnished by the Massachusetts Antitoxin and Vaccine Laboratory.

RESULTS

Effect of sulfanilamide on the pneumococcal action of blood in non-pneumonic subjects (Table I) Defibrinated blood of 9 subjects was tested. In 6 instances the sulfanilamide was added to the blood *in vitro* in amounts sufficient to make the desired concentration and tested simultaneously with the control blood containing no sulfanilamide. In the 3 remaining subjects a second blood was taken after each had received 10 grams of the drug in divided doses (4 hours after the last dose). The concentration of the unconjugated sulfanilamide in the second blood was 7.6 to 8.5 mgm per 100 cc.

As was to be expected, most of the subjects had some pneumococcal power in the control blood. Growth or killing in these bloods was complete.

TABLE I
Effect of sulfanilamide on the phagocytic and pneumococcal activity of defibrinated blood against Type III pneumococci in persons without pneumonia

Subject	Control tests without sulfanilamide			Tests with sulfanilamide					
	Phagocytosis		Pneumococci killed per 0.5 cc of blood†	Concentration of free sulfanilamide	Phagocytosis		Inhibition of growth (absence of color change)		Pneumococci killed per 0.5 cc of blood
	Average number of diplococci per polymorphonuclear leukocyte	Per cent of polymorphonuclear leukocytes phagocytizing			Average number of diplococci per polymorphonuclear leukocyte	Per cent of polymorphonuclear leukocytes phagocytizing	24 hours	48 hours	
McD	0	0	0	10.0	0.06	6	10^1	10	0
M	0.1	8	0	5.0	0.04	4	10	0	0
				10.0	0.02	2	10^1	10^2	0
				15.0	0.02	2	10^2	10^2	0
				20.0	0	0	10^2	10^1	0
V	0	0	10^2	20.0	0	0	10^3	10^2	10^2
Br	0.06	6	10^1	15.0	0.06	4	10^2		10^2
R	0.06	6	10^1	10.0	0.01	4	10^2		10^2
F	0	0	10^2	10.0	0.06	4	10^2		10^2
L	0.04	4	0	7.6*	0.03	2	10^1		0
B	0.6	32	10^2	8.5*	0.40	28	10^2		10^1
M	0.1	10	10^1	8.3*	0.10	8	10^2		10^1

* Blood taken after 10 grams were given by mouth during previous 24 hours. In others, sufficient sulfanilamide was added to the blood in 0.1 cc. of saline (*in vitro*) to make the desired concentration.

† Color change (growth inhibition) at 24 and 48 hours corresponded in each instance.

within 24 hours. In the 3 subjects lacking pneumococidal activity the addition of sulfanilamide in amounts to make 7.6 or more mgm per 100 cc. resulted in definite inhibition of growth for 24 hours with inocula up to 10,000 organisms. Larger concentrations of the drug increased and prolonged the bacteriostatic effect. No bactericidal effect was noted. In the subjects possessing pneumococidal activity in the control blood the addition of sulfanilamide enhanced this property only slightly or not at all. Practically no phagocytosis was observed in any of the control tests. The addition of sulfanilamide had no effect on phagocytosis.

Patients possessing pneumococidal activity before treatment (Table II). There were 11 patients in whom the initial blood taken before treatment was bactericidal for Type III pneumococci.

In only one instance (D. M.) was this property associated with demonstrable agglutinins and mouse protective antibody. This patient was also the only one with blood exhibiting any appreciable phagocytic activity before treatment. Crisis occurred within 24 hours after only 4 grams of the drug had been given. *In vitro* tests performed on the preliminary bloods of the 11 patients after addition of sulfanilamide gave results similar to those obtained in normals. The addition of specific serum increased the pneumococidal activities of these bloods.

Sulfanilamide alone was used in the treatment of 7 of these patients. Later bloods showed the same or greater pneumococidal power. Agglutinins and mouse protective antibodies were demonstrated in subsequent serums in 6 of these cases. Two of these patients died, one (P. F.) on the

TABLE II
Patients with Pneumococcus Type III pneumonia whose blood was bactericidal for Type III pneumococci before treatment was begun

Name	Sex	Age	Results of blood cultures*	Day treatment begun	Pneumococidal action before treatment†	Treatment	Termination of disease		Remarks
							Mode	Day	
P. F.	M	65	Pn. III—6 Neg.—7, 8 S aureus— 9, 13	8	10 ⁴	Sulfanilamide	Death	13	Death apparently due to <i>S. aureus</i> sepsis after developing agglutinins and mouse protective antibodies for Pn. III.
J. R.	M	67	Neg.—3, 4 A‡	3	10 ⁴	Serum	Death	7	Extended after treatment in spite of good titer of agglutinins and protective antibody; Pn. III, <i>S. Armatylosus</i> and <i>S. aureus</i> in lungs at autopsy.
A. F.	M	5	Neg.—6, 7 A‡	6	10 ⁴	Sulfanilamide	Death	14	Lungs = <i>S. Armatylosus</i> , <i>S. aureus</i> and <i>H. influenzae</i> (no Pn.). No agglutinins or protection on 8th day (last test).
C. N.	F	43	Neg.—5, 7 9, 10, 11 Pn. III—8	9	10 ⁴	Sulfanilamide	Lysis	9-10	Blood culture sterile before first dose had agglutinins and protection 2 days later.
D. M.	F	16	Neg.—2 (7)	3	10 ⁴	Sulfanilamide	Crisis	4	Agglutinins and protection present on third day (Pneumonia may have begun 4 days earlier).
J. A.	M	18	Neg.—5, 6	3	10 ⁴	Sulfanilamide	Crisis	8	Developed protective antibody and agglutinins.
T. P.	F	53	Neg.—4, 5 6, 7	4	10 ³	Sulfanilamide	Crisis	5	Developed protective antibody and agglutinins.
R. W.	F	41	Neg.—3, 4, 5	3	10 ⁴	Sulfanilamide	Crisis	8	Developed protective antibody and agglutinins.
E. S.	F	55	Neg.—2, 3, 4, 7	3	10 ⁴	Serum and sulfanilamide	Lysis	8+	Transient balance of protective antibody after treatment.
R. G.	F	43	Neg.—3, 4, 5, 7, 11, 12	4	10 ⁴	Serum and sulfanilamide	Crisis	8	Nonprotein nitrogen = 48, 90, 55 and 35 mgm per 100 cc. on Days 4, 7, 11 and 16, respectively. Balance of agglutinins and protection maintained.
M. N.	M	60	Neg.—1, 2	1	10 ⁴	Serum and sulfanilamide	Crisis	2	Balance of agglutinins and protection established and maintained.

* Numbers represent days of the disease.

† Number of diplococci killed in 0.5 cc. of blood.

‡ This is the only patient with appreciable phagocytic titer before treatment (1.88 diplococci per polymorphonuclear leukocyte).

‡ A = Autopsy.

13th and the other (A F) on the 14th day. The former had a positive blood culture for Type III pneumococcus on the sixth day but 2 subsequent cultures taken prior to treatment were sterile. Following treatment the patient developed *Staphylococcus aureus* bacteremia and died. In Patient A F no tests were done after the 8th day. Blood cultures before and after the beginning of treatment and at autopsy were all sterile. The lungs showed bronchopneumonia and cultures showed hemolytic streptococci, *Staphylococcus aureus*, and influenza bacilli, but no pneumococci. One of the patients who recovered (C N) had a positive blood culture on the eighth day but the culture taken the next day just before treatment was begun was sterile.

Specific antiserum alone or with sulfanilamide was used in the treatment of the remaining 4 patients. All had negative blood cultures throughout and developed and maintained a balance of agglutinins and protective antibody in the blood after treatment. One of these patients (J R) died. He received no sulfanilamide, and the lesion in the lung extended after serum administration in spite of negative blood cultures and a balance of agglutinins and mouse protective antibodies. Type III pneumococci were recovered from both lower lobes and, in addition, hemolytic streptococci and *Staphylococcus aureus* were cultured from one of the lobes.

In general, therefore, patients with pneumococcal activity in their blood during the disease and before treatment developed homologous type-specific agglutinins and mouse protective antibodies following sulfanilamide therapy and either recovered or died with secondary infections associated with other organisms. The only patient in whom such antibodies were not demonstrated was A F. In this patient no tests were done after the eighth day and death associated with a superinfection occurred 6 days later, during which time specific antibodies may have developed. In the 2 bacteremic patients (A F and C N) the pneumococcal infection was apparently overcome at the time treatment with sulfanilamide was instituted. Following treatment with specific serum, a balance of agglutinins and protective antibodies was readily established.

Patients lacking pneumococcal activity in their blood before treatment (Table III) In 15 of the

26 patients tested, the defibrinated blood taken before treatment failed to kill any Type III pneumococci or killed only the smallest number (4 to 10 organisms) inoculated into 0.5 cc of the blood. Phagocytosis, agglutinins, and passive protection of mice could not be demonstrated in any instance.

In vitro tests of the effect of serum and sulfanilamide were carried out with the blood taken before treatment. The addition of sulfanilamide to a final concentration of 1:10,000 (10 mgm per 100 cc) resulted in marked bacteriostasis in 12 of 14 cases tested. Growth inhibition was apparent at 24 hours with inocula of 100,000 and 1,000,000 organisms and was still effective with the same or slightly smaller inocula after 48 hours' incubation in 10 of the tests. This bacteriostatic effect was manifest even in bloods from which pneumococci were cultured in large numbers (Cases D B and M W). Actual killing of some pneumococci after the addition of sulfanilamide occurred in 3 instances (and possibly in 4 others in whom blood agar streak plates showed no growth but pour plates were not made with the blood containing the smaller inocula), but it was always of low grade. Only 100 or 1000 pneumococci were killed in these bloods. Phagocytosis was not enhanced by the addition of sulfanilamide. In Case M W the addition of sulfanilamide to a concentration of 15 or 20 mgm per 100 cc resulted in somewhat greater inhibition, and 10,000 and 100,000 pneumococci, respectively, were killed. In the same blood, a concentration of 5 mgm per 100 cc of sulfanilamide had no effect.

The addition of therapeutic rabbit serums to a final dilution of from 1:60 to 1:300 resulted in the killing of from 100 to 100,000 pneumococci in 0.5 cc of the blood of all but 4 of the patients. In 3 of the latter 4 cases there was heavy blood stream invasion. In some instances free growth of organisms was inhibited for the first 24 hours in the blood containing large numbers of inoculated pneumococci. The addition of the same amount of serum together with sulfanilamide to a concentration of 10 mgm per 100 cc. resulted quite regularly in an increased bacteriostatic and bactericidal action as compared with either serum or sulfanilamide alone. Phagocytosis was definitely enhanced only in the presence of serum, and the addition of sulfanilamide produced no added effect. In the phagocytic mixtures contain-

ing the larger amounts of serum, the results were often obscured by the agglutination of pneumococci and of leukocytes and the injury to the latter in making the smears. Thus rendered the satisfactory estimation of phagocytosis impossible.

In summary, the addition of sulfanilamide to the blood of patients lacking bactericidal action resulted fairly regularly in moderate to marked bacteriostasis. Occasionally there was some bactericidal action but no phagocytosis occurred. Immune serum on the other hand, induced bactericidal action and frequently phagocytosis as well. The combination of serum and sulfanilamide resulted in marked bacteriostasis and better bactericidal action than when either was used alone in the same amount.

Tests were done *after treatment* with serum alone in 4 cases, after the administration of sulfanilamide alone in 2 cases, and after both had been given in 8 cases. The results may be summarized for each of these 3 groups.

After serum alone. One patient, R. F., received 40 cc. of serum on the fourth day after which no antibodies could be demonstrated by any of the tests. The blood culture before treatment was sterile but those taken on the next 2 days showed large numbers of Type III pneumococci and the patient died. The addition of serum to his blood before treatment failed to induce pneumococcal activity. The second patient, F. S., received 150 and 60 cc. of serum, respectively, on the sixth and seventh days following which the blood showed marked pneumococcal action, irregular agglutinins and mouse protective antibodies, and no phagocytosis. Sulfanilamide was administered during the next 2 days but no further tests were done. Blood cultures were negative before and after treatment, and the patient died. Patients J. T. and E. J. received 60 and 100 cc. of antiserum respectively on the fifth day of the disease and recovered. Pneumococcal activity and mouse protective antibody were present after this treatment and there was transient appearance of agglutinins and phagocytosis.

After sulfanilamide alone. Both patients A. S. and Ma. McC., recovered without developing demonstrable antibodies. Blood cultures were negative in each case. The blood of Ma. McC. showed growth inhibition when the concentration of free sulfanilamide was 6.9 but not at the lower

level of 2.1 mgm. per cent. The pneumonia in Patient A. S. was atypical with only slight pulmonary involvement.

After serum and sulfanilamide. Patient M. McC. received sulfanilamide for 2 days during which his blood showed only growth inhibition. Following the administration of serum and further doses of sulfanilamide sufficient to raise the blood concentration of the drug there was some pneumococcal activity. There was a transient balance of mouse protective antibody but no agglutinins or phagocytosis could be demonstrated and the patient died. Blood cultures were positive before and sterile after serum treatment. Four other patients in this group died. Two of them, F. I. and F. M., showed only growth inhibition in their blood but no bactericidal action, phagocytosis, agglutinins, or mouse protective antibody. The other 2 each developed a good balance of antibodies measurable by all of the tests. One of them, C. S., developed a hemiplegia on the second day after an apparent crisis and died 2 days later; the other had a prolonged course and died on the 24th day. All 5 of these fatal cases had Type III pneumococcus bacteremia. In Patient C. S., no pneumococci could be cultured at autopsy and in Patient M. W. there were multiple abscesses in the lung which yielded Type III pneumococcus and *Staphylococcus aureus* but blood cultures taken after treatment were sterile.

In two non bacteremic patients L. T. and J. S. prompt crisis occurred following treatment, and antibodies were demonstrable by all the tests.

The last patient, J. K., is of especial interest. He received 2 cc. of antiserum on the second day. No further serum was given because of a severe chill with rise in temperature to 107° F. Blood cultures before this dose and on the following day before sulfanilamide therapy was begun were both positive for Type III pneumococcus. Following treatment with the drug the blood exhibited a bacteriostatic action and on one occasion also showed some bactericidal activity. No other antibodies could be demonstrated before or later although the patient recovered by crisis on the day after sulfanilamide therapy was begun.

In summary, when a balance of antibody demonstrable by agglutination, mouse protection or phagocytosis was established and maintained, the patients in general, at

[illegible]

TABLE III —Continued

Patient	Sex	Age	Blood culture		Before treatment						After treatment						Termination of disease		Remarks						
			Bacilli	Days	Day of disease	Added in vitro		Growth inhibition		Pneumococci killed	Phagocytic index	Day of disease	Previous therapy		Sulfanilamide	Growth inhibition		Pneumococci killed		Phagocytic index	Agglutination	Blood protection			
						Sulfanilamide	(dilution)	24 hours	48 hours				Sulfanilamide	Specific serum		Free	Total						24 hours	48 hours	
																									mgm. per 100 ccs.
E. J.	M	60	—	2, 3, 5, 8, 10	3	0	0	0	0	0	5	100	0	10 ¹⁰	10 ¹⁰	10 ¹⁰	3.74	8	10 ¹⁰	8	10 ¹⁰	Lysis	8+	Irregular protection tests	
L. T.	F	46	—	3, 4, 9, 10	5	0	0	0	0	0	6	80	0	10 ¹⁰	10 ¹⁰	10 ¹⁰	0.18	0	10 ¹⁰	0	10 ¹⁰	0	10 ¹⁰	Crisis	6
J. S.	M	44	—	5, 6, 14, 17, 18	5	0	0	0	0	0	7	100	0	10 ¹⁰	10 ¹⁰	10 ¹⁰	3.00	32	10 ¹⁰	10 ¹⁰	10 ¹⁰	10 ¹⁰	10 ¹⁰	Crisis	7
J. K.	M	61	—	1, 4, 5, 10, 11, 2, 3	2	0	0	0	0	0	2	20	5.3	10 ¹⁰	10 ¹⁰	10 ¹⁰	0.02	0	10 ¹⁰	0	10 ¹⁰	0	10 ¹⁰	Crisis	4
A. S.	M	58	—	3, 4, 5, 9	3	0	0	0	0	0	2	20	5.3	10 ¹⁰	10 ¹⁰	10 ¹⁰	0.38	8	10 ¹⁰	0	10 ¹⁰	0	10 ¹⁰	Crisis	4
Mrs. McC.	F	69	—	6, 7, 8, 9	10	0	0	0	0	0	12	0	0	10 ¹⁰	10 ¹⁰	10 ¹⁰	0.10	0	10 ¹⁰	0	10 ¹⁰	0	10 ¹⁰	Lysis 10-12	Sulfanilamide begun 3d day discontinued 12th day Sulfanilamide given only on 3d day Mild bronchopneumonia

* Level of free sulfanilamide after 8 grams on 4th day

† Explanations: In tests done before treatment, sulfanilamide and serum to make the required final concentration were contained in 0.1 cc of physiological saline and added to each 0.5 cc of dehydrated blood.

Growth inhibition: the numbers represent the largest number of pneumococci which failed to change the color of blood after incubation.

Pneumococci killed: the numbers represent the largest number of pneumococci which failed to yield any growth after 48 hours incubation followed by culture in blood agar pour plates and on the surface of blood agar plates (and occasionally also in rabbit blood broth).

Phagocytic index = average number of pneumococci per polymorphonuclear leukocyte

Previous therapy = total amount given prior to the time blood was taken

Pn. III = Pneumococcus Type III

A = autophag

Neg = negative (no growth)

N.P.N. = nonprotein nitrogen (expressed in mgm. per cent)

† 8 grams sulfanilamide given only on the 3rd day (after this test)

† Serum discontinued after 2 cc gave chill and temperature of 107° F on 2d day

the pneumococcal infection. If death occurred it was associated with complications of the pneumonia or with conditions not directly related to the pneumococcal infection. In 3 patients treated with sulfanilamide alone recovery was not associated with demonstrable antibodies. One of these patients had mild atypical pneumonia and 2 had sterile blood cultures. The third had bacteremia and received a small dose of serum which had no apparent effect on the immune status, either by virtue of the antibody or because of the elevated temperature that resulted (15), since bacteremia was still demonstrated on the day after the high fever and no antibodies were found.

Three other patients are of interest but are not included in the table because pneumococcal tests were not carried out. The first was a 47-year-old man who started on sulfanilamide therapy on the sixth day. No agglutinins or mouse protective antibodies could be demonstrated following treatment, and the patient died on the 13th day. Blood cultures taken before treatment were sterile and later ones were positive. The second patient was a boy of 17 who received 40 cc. of specific antiserum on the third day after which agglutinins (1:32) and mouse protection (against 1,000,000 fatal doses in 0.2 cc. of serum) were demonstrated in his blood and there was a prompt crisis within 6 hours of the time the first dose was given. Preliminary blood culture was sterile. The third patient, a man 66 years old, had a negative blood culture on the third day. On the following day sulfanilamide therapy was begun and the blood culture taken before the first dose was positive for Type III pneumococcus. Subsequent blood cultures were sterile, agglutinins (1:4) and protection (against 1000 fatal doses) were demonstrated in the patient's serum on the seventh day and later, and he recovered.

DISCUSSION

The results of the present studies indicate that in human blood, sulfanilamide, either when added *in vitro* or when absorbed after ingestion, inhibits the free growth of large numbers of virulent Type III pneumococci. Concentrations of about 7 mgm per 100 cc. or higher are apparently necessary for this bacteriostatic action. The drug usually has no bactericidal effect on pneumococci of

this type even in concentrations up to 20 mgm per cent. The same growth inhibition occurs in the blood of non-infected individuals and in the blood of patients with Pneumococcus Type III pneumonia during the acute disease. Neither this growth inhibition nor the bactericidal activity when it occurs are associated with any marked degree of phagocytosis as demonstrated by the test employed here. Type-specific antipneumococcal rabbit serums usually enhance the pneumococidal power of blood and this activity is usually associated with phagocytosis. The combination of sulfanilamide and type-specific serum is more effective than either of these agents used separately in promoting bacteriostasis and pneumococidal activity in the fresh defibrinated blood of patients during the acute disease.

It is not surprising that bactericidal activity was not induced with specific antiserum in the blood of some patients acutely ill with Type III pneumococcus pneumonia. In the first place, the potency of the serums used was unknown and was frequently low. These therapeutic Type III rabbit serums were among the early lots produced and experimental methods were used in immunization of the animals and in concentration of the serums. Also, no attempts were made to rule out any prozone phenomenon in the tests (6b). The patients in whose blood the antisera failed to induce bactericidal action were heavily infected and may have required relatively large amounts of antibody to neutralize the antibactericidal substances presumably present in the blood. This factor was not controlled in the present studies. The leukocytes were not at fault quantitatively, since adequate numbers were found in the defibrinated blood at the time of each test. It is not possible to rule out functional failure of the leukocytes either as a result of the disease or through the action of the carbohydrate-anticarbohydrate combination present in the blood at the time of the tests (16). The more recent lots of rabbit serums that we have had available have been more potent in terms of units and more effective both in the test tube experiments and in the regularity with which a balance of antibodies could be established in the treated patient.

The phagocytic test as employed here is a rather severe one and it is not surprising that this test correlated better with the presence of heat-stable

antibodies (agglutinins and mouse protective antibody) than with the more sensitive pneumococidal test. The inoculum in 0.5 cc. of blood contained, in addition to approximately 100,000,000 pneumococci, 0.1 cc. of the original culture fluid. This contained a considerable amount of specific antibactericidal material (6b) in the form of the capsular polysaccharide. It was necessary to have enough antibody to neutralize the action of the carbohydrate and a sufficient excess to promote phagocytosis. Agglutination interfered with the *in vitro* tests but the phagocytosis was usually discernible in mixtures in which this reaction occurred.

It appears from the findings presented that recovery from Type III pneumococcus pneumonia, as in other types is usually associated with the development of heat stable antibodies demonstrable by agglutinins mouse protection and by phagocytosis in fresh blood. Occasional patients did recover however without demonstrable antibodies. In view of the frequency with which the Type III pneumococcus is found in the normal nasopharynx and particularly in the nasal secretions and the sputum of persons with chronic respiratory infections, the possibility arises that in such cases the finding of this organism is only incidental and that it is not the true instigator of the pneumonia in the given case. In 2 of the patients (Ma McC. and A S), who recovered without developing type-specific antibodies this possibility can not be ruled out since there was only a mild atypical pneumonia in one, blood cultures were sterile in both, and the Type III pneumococci were obtained only from purulent sputum in each instance. However in the third patient J K. bacteremia was present and yet recovery occurred without antibodies. To be sure, there was some bactericidal activity on one occasion after recovery at a time when the concentration of sulfanilamide was low, but no other antibodies were found. This suggests that in these cases either recovery took place without the development of heat stable antibodies or that such antibodies were only transient so that they appeared and disappeared between the times the tests were made, or antibodies developed against the homologous strain but could not be demonstrated with the stock strain used. The two latter explanations need not necessarily be invoked in view of the known vari-

ability with which different animals respond to immunization with Type III pneumococci (2).

It may be inferred from the present findings that whatever therapeutic activity sulfanilamide may have is probably due to its bacteriostatic effect. If the patient had pneumococidal properties in his blood this effect may be sufficient to keep the infection localized until the natural defenses are mobilized either through the development of heat stable antibodies or by whatever mechanism may be involved when recovery occurs without such antibodies. Obviously specific antiserum should hasten such recovery unless the antigen-antibody combination can occur and produce toxic effects *in vivo*, or unless quantitative factors such as those operative in the prozone phenomenon can occur within the patient. Such effects have not been demonstrated but the possibility that they may be responsible for some of the immediate untoward effects of serum therapy might be considered. If these effects do occur it should be possible to avoid them by controlling the speed of administration of antibody in the severely infected patient. However it is not proper to infer from the closed system involved in the test tube experiment that these ill effects take place in the circulating blood or tissues of the patient.

If the patient fails to develop antibodies or to receive such antibodies passively, blood invasion may occur in spite of sulfanilamide therapy and death may ensue. A consideration of some of the pathological features (17, 18) and of some of the host factors (17, 19) frequently involved in cases of Pneumococcus Type III pneumonia leaves room for adequate causes of death even when the pneumococcal infection itself is apparently overcome. Deaths in such cases may be due to systemic diseases to late complications of the pneumonia or to super infections with other organisms. All of these conditions have been noted in the present group of cases.

The failure of macrophages to appear in the consolidated lung has been offered as an explanation of the local spread of the disease and of death in patients who have circulating antibodies (20). Such a mechanism may have been involved in one of the present cases (J R). In this patient pneumococidal activity was present in the blood before treatment with specific serum. Following treat-

ment the lesion extended to the opposite lung, in spite of the fact that the bactericidal action persisted and a balance of agglutinins and mouse protective antibodies was established and maintained and blood cultures were sterile both before and after treatment. Microscopic sections of the consolidated lobes of this patient revealed only occasional macrophages, and polymorphonuclear leukocytes predominated in the exudate.

Further studies with sulfanilamide and sulfa-pyridine are being continued with Drs. Spring and Lowell during the present season.

CONCLUSIONS

Sulfanilamide in concentrations of 7 mgm or more per 100 cc inhibits the growth of large numbers of Type III pneumococci in the blood of non-pneumonic individuals or of patients ill with pneumonia due to this organism when such bloods lack pneumococidal activity. The drug probably does not influence phagocytosis in these bloods. It usually exerts no bactericidal effect in a concentration of 10 mgm per 100 cc, but may do so in greater concentrations.

Patients with *Pneumococcus* Type III pneumonia, whose blood is bactericidal for pneumococci of the homologous type during the acute disease and before treatment, usually acquire homologous type-specific agglutinins, mouse protection, and phagocytosis after treatment with either sulfanilamide or serum or both. Blood invasion does not occur after treatment in such cases and if death occurs it is usually due to superinfections or to other conditions not directly related to the Type III pneumococcal infection. In an occasional patient the pneumonia extends in spite of the presence of circulating antibodies and in spite of the absence of bacteremia throughout the disease.

Therapeutic antipneumococcal rabbit serums induce pneumococidal activity in the blood of patients ill with pneumonia due to this type. Antiserum and sulfanilamide used together have a greater bacteriostatic and bactericidal effect than the same amounts of either the serum or the sulfanilamide used separately. The bactericidal-promoting property of the antiserum is usually accompanied by demonstrable phagocytosis.

In patients whose blood lacks bactericidal properties, treatment with sulfanilamide probably ren-

ders the blood bacteriostatic until heat stable specific antibodies (agglutinins and mouse protection) develop or until a balance of such antibodies is passively introduced. When such heat stable antibodies are acquired, the pneumococcal infection is usually overcome. With antisera in proper amounts, the infection may be overcome without the additional use of sulfanilamide, especially in patients who are not heavily infected. Death in either event may nevertheless occur, but under such circumstances it is due either to complications or to conditions not related to the Type III pneumococcal infection.

Following treatment with sulfanilamide alone, occasional patients with Type III pneumococcus pneumonia recover without developing demonstrable homologous type-specific antibodies. This may occur even if the pneumococcus is recovered from the blood stream.

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BIBLIOGRAPHY

- 1 Finland, M., and Brown, J. W., Treatment of *Pneumococcus* Type III pneumonia with specific serum and sulfanilamide. *New England J. Med.*, 1939, 220, 365.
- 2 See Finland and Winkler (3b, Reference 1).
- 3 (a) Finland, M., and Sutcliffe, W. D., Specific cutaneous reactions and circulating antibodies in the course of lobar pneumonia. *J. Exper. Med.*, 1931, 54, 637.
- (b) Finland, M., and Winkler, A. W., Antibody response to infections with Type III and the related Type VIII pneumococcus. *J. Clin. Invest.*, 1934, 13, 79.
- 4 (a) Finland, M., and Dowling, H. F., Cutaneous reactions and antibody response to intracutaneous injections of pneumococcus polysaccharides. *J. Immunol.*, 1935, 29, 285.
- (b) Finland, M., and Rueggsegger, J. M., Immunization of human subjects with the specific carbohydrates of Type III and the related Type VIII pneumococcus. *J. Clin. Invest.*, 1935, 14, 829.
- 5 (a) Ward, H. K., Observations on the phagocytosis of the pneumococcus by human whole blood. I. The normal phagocytic titer and the anti-phagocytic effect of soluble specific substance. *J. Exper. Med.*, 1930, 51, 675.
- (b) Sutcliffe, W. D., and Finland, M., Antipneumococcus immunity reactions in individuals of different ages. *J. Exper. Med.*, 1932, 55, 837.

- (c) Robertson O H. and Cornwell M A. A study of the resistance of normal human beings to recently isolated strains of pathogenic pneumococci. *J Exper Med.*, 1930 52 267
6. (a) Sia, R. H. P., Studies on pneumococcus growth inhibition. VI The specific effect of pneumococcus soluble substance on the growth of pneumococci in normal serum leukocyte mixtures. *J Exper Med.*, 1926 43, 633
- (b) Ward H K., An examination of the mechanism of pneumococcus immunity by means of bactericidal measurements I. The reaction between the anti carbohydrate antibody and the purified specific carbohydrate. *J Exper Med.*, 1932, 55 511
- II. The reaction between anticarbohydrate antibody and type-specific products of the organism. *J Exper Med.*, 1932, 55 519
- (c) Ward, H. K., and Enders J F., An analysis of the opsonic and tropic action of normal and immune sera based on experiments with the pneumococcus. *J Exper Med.*, 1933 57 527
- 7 Robertson, O H., Terrell, E. E., Gracer J B., and Cornwell, M. A., The relation of natural humoral antipneumococcal immunity to the inception of lobar pneumonia. *J Exper Med.*, 1930 52 421
8. (a) Domag, G., Ein Beitrag zur Chemotherapie der bakteriellen Infektionen. *Deutsche med. Wchnschr.*, 1935 61 256.
- (b) Rosenthal S M Studies in chemotherapy III The effects of p-aminobenzene sulphonamide on pneumococci *in vitro* *Pub Health Rep* 1937 52, 192
- 9 (a) Rosenthal S M Studies in chemotherapy II. Chemotherapy of experimental pneumococcus infections. *Pub Health Rep.* 1937 52, 48.
- (b) Cooper F B. Gross P., and Mellon R. R., Action of p-aminobenzenesulfonamide on Type III pneumococcus infections in mice. *Proc. Soc. Exper Biol and Med.*, 1937 36, 148.
- (c) Gross P., and Cooper F B., Efficacy of p-aminobenzenesulfonamide in experimental Type III pneumococcus pneumonia. *Proc. Soc. Exper Biol and Med.*, 1937 36, 225
- 10 (a) Heinzelman, J H. Hadley P B., and Mellon, R. R., The use of p-aminobenzene sulfonamide in Type III pneumococcus pneumonia *Ann. J M. Sc.*, 1937 193 759
- (b) Sadusk, J F., Observations on sulfanilamide therapy in pneumonia and meningitis due to Type III pneumococci. *New England J Med.* 1938 219 787
- 11 (a) Bullowa J G M., The Management of the Pneumonias Oxford University Press, New York, 1937 p. 201
- (b) Bullowa J G M., Correspondence quoted in Mellon, R. R., Gross P and Cooper F B., "Sulfanilamide Therapy of Bacterial Infections." Charles C. Thomas Springfield Ill., and Baltimore, 1938, p 206
12. (a) Neufeld, F., and Etinger Tulczynska, R., Bakterienkapseln und Quellungsreaktion. *Ztschr f Hyg u. Infektionskr.*, 1933 114 769
- (b) Sabin, A. B., Immediate pneumococcus typing directly from sputum by the Neufeld reaction. *J A. M. A.*, 1933 100 1584
- 13 Finland M., and Sutliff W D., Immunity reactions of human subjects to strains of pneumococci other than Types I II, and III *J Exper Med.*, 1933 57 95
- 14 Marshall, E. K. Determination of sulfanilamide in blood and urine. *J Biol. Chem.* 1937 122 263
- 15 (a) Enders J F., and Shaffer M F., Studies on natural immunity to pneumococcus Type III. I The capacity of strains of pneumococcus Type III to grow at 41 C. and their virulence for rabbits *J Exper Med.* 1936 64 7
- (b) Rich A. R., and McKee, C. M., The mechanism of a hitherto unexplained form of native immunity to the Type III pneumococcus. *Bull. Johns Hopkins Hosp.*, 1936, 59 171
16. Cornwell, H. W and Centeno J A., The reaction of the white blood cells to specific precipitates. *J Immunol.*, 1929 17, 53.
- 17 Finland, M., and Sutliff W D., Infections with Pneumococcus Type III and Type VIII. *Arch. Int. Med.*, 1934, 53, 481
- 18 Finland M., Brown, J W., and Rueggesser J M., Anatomic and bacteriologic findings in infections with specific types of pneumococci including Types I to XXXII *Arch Path.*, 1937 23 801
- 19 Blake, F G Observations on Pneumococcus Type III pneumonia. *Ann. Int. Med.*, 1931 5 673
- 20 Robertson, O H., Recent studies on experimental lobar pneumonia. Pathogenesis, recovery and immunity *J A. M. A.*, 1938 111, 1432.

THE EFFECTS OF SPINAL ANESTHESIA ON THE CIRCULATION IN NORMAL, UNOPERATED MAN WITH REFERENCE TO THE AUTONOMY OF THE ARTERIOLES AND ESPECIALLY THOSE OF THE RENAL CIRCULATION

By H. W. SMITH, E. A. ROVENSTINE, W. GOLDRING, H. CHASIS AND H. A. RANGES

(From the Departments of Physiology, Anesthesia and Medicine, New York University College of Medicine and the Third (New York University) Division of Bellevue Hospital, New York City)

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The present study comprising observations on 21 subjects given high spinal anesthesia, is an investigation of the effects of anesthetic denervation in normal man uncomplicated by surgical intervention, on the circulation with particular reference to the vascular bed of the kidney. It is concluded that the renal arterioles are distinctly autonomous, in the sense that under basal conditions the renal vascular tone is not affected by anesthetic denervation. Our observations further suggest that the arteriolar bed generally (apart from the skin) possesses considerably more autonomy than is usually attributed to it—sufficient, in fact, in the normal supine individual at rest, to maintain an essentially normal arterial pressure. We find no evidence of significant arteriolar dilatation during high spinal anesthesia such reduction in blood pressure as occurs being attributable, we believe, to diminished circulating blood volume in consequence of dilatation of the capillaries, venules, and veins.

Part I deals with the following topics: (1) Methods, (2) the effects of spinal anesthesia on renal blood flow, (3) on the arterial pressure, and (4) on the reflex responses to posture, hypercapnia, and anoxemia. Part II consists of a review of the evidence on (5) the existence of tonic vasoconstrictor activity and on (6) the existence of autonomy in the peripheral arterioles generally, and (7) in the kidney in particular, and (8) the peripheral effects of hypercapnia and anoxemia. In (5) it is brought out that the notion of tonic vasoconstrictor activity in the sympathetic nervous system apart from the skin is based largely upon animal experiments which are seriously complicated by general anesthesia, venous dilatation, etc., and that the information so obtained cannot be

transferred with confidence to normal animals, and certainly not to man.

PART I

1. Methods

The subjects were male convalescent patients ranging in age from 18 to 50 years who, with a single exception, presented no abnormal signs contraindicating selection for this study. They were examined in the morning in the basal, fasting condition, and were prepared for the measurement of renal blood flow and filtration rate by the clearance method, as described by Smith, Goldring and Chasis (93). In the earlier observations the phenol red and inulin (35) clearances were followed, but after the introduction of the diodrast clearance (93) all three clearances were used. The phenol red clearance serves as a check on the diodrast clearance, the constancy of the phenol red/diodrast clearance ratio before and after an esthesia demonstrating that procaine *per se* has no effect upon the tubular excretory mechanism. The infusions corresponded to the typical infusion cited by Chasis, Ranges, Goldring and Smith (18). Zero time was taken as the beginning of the priming infusion which occupied about 10 minutes; the infusion was then changed to the sustaining infusion, the first urine collection period being started at about 30 minutes. The sustaining infusion was usually interrupted momentarily to permit injection of the anesthetic, and a short washout period was allowed to reestablish blood levels of diodrast, etc. before the next urine collection period was started. The urine collection periods (10 to 15 minutes in length) were timed to 15 seconds and all urine samples were collected by catheter with sterile precautions, the bladder being washed out with 20 cc. of saline. (In our opinion, single urine collection periods obtained by voluntary voiding or even by catheterization without rinsing the bladder may be highly inaccurate; we have made nearly 3000 catheterized and rinsed collections, and we recognize that it is impossible even by this method to empty the bladder completely every time.) All our observations have been made on the descending limb of water diuresis, and to prevent an excessive reduction of urine flow we have

usually incorporated 20 per cent Na SO₄ in the infusion fluid. Analyses were carried out as described by Smith, Goldring, and Chasis (93), except that phenol red was determined on an Evelyn colorimeter, using a Number 540 filter.

Anesthesia was induced by an experienced anesthetist (E. R.) by the intrathecal injection between L3 and L4 (after local anesthesia of the skin) of procaine crystals (= novocaine) dissolved in 2 to 5 cc. of spinal fluid. No premedication was given. The quantities of procaine used were somewhat larger than the usual surgical dose, since we wished to obtain maximal anesthesia. In earlier instances the injection was made with the subject in the lateral position, after which he was immediately turned to the supine and tilted at an angle of -20° to -10° until the highest level of anesthesia was established. However, it is impossible by this method to be certain that the anterior roots are exposed to maximal concentration of the anesthetic, and consequently in later observations the subject was injected in the prone position and left lying on his face until anesthesia had reached its highest point (5 to 10 minutes), after which he was turned on his back. Blood pressures were taken by auscultation of the brachial artery at frequent intervals. The level of anesthesia was conservatively determined by sensory stimulation, the dermatomes being designated according to the recent description of Foerster (28). Complete loss of all sensation up to and including the umbilicus was taken to indicate anesthesia of the posterior roots up to and including T12, to the xiphoid process, T7, to just below the nipple, T6, to just above the nipple, T5, to the clavicle, T2, to the inner aspect of the forearm, T1, to the hollow above the clavicle and the outer shoulder, C4, to the neck, C3.

2 The effects of anesthesia on renal blood flow

Reflex oliguria and renal ischemia The discussion of the effects of spinal anesthesia upon the renal circulation must be prefaced by brief mention of the reflex effects of spinal puncture *per se* on urine flow and renal blood flow. Spinal puncture is frequently accompanied by an abrupt reduction in urine flow and less frequently by a marked but transient reduction in renal clearances. We have noted that frequently, though not invariably, the urine flow has fallen abruptly at the time of puncture and remained at relatively low levels (0.5 to 2.5 cc. per minute) thereafter. Theobald and Verney (100) have shown in dogs that trauma of the vertebral periosteum causes an inhibition of urine excretion in the denervated kidney, they have interpreted this phenomenon to be a result of an increased secretion of the antidiuretic hormone in consequence of reflex excitation of the posterior pituitary gland, and it

is probable that this explanation applies to those instances in our observations where the urine flow fell sharply at the time of puncture without a simultaneous change in clearances. We have never observed an increase in urine flow in consequence of spinal anesthesia, although not specifically designed to examine this question, we believe that our observations would have revealed any tendency for diuresis to occur, were such a tendency present. One of us has argued elsewhere (92) that "denervation diuresis" is a phenomenon which is discoverable only in the anesthetized animal and our present observations lead us to affirm that anesthetic denervation of the kidneys has no specific effect on water excretion.

In a few instances the diodrast, phenol red, and inulin clearances have fallen markedly at the time of spinal puncture, suggesting constriction of the renal arterioles in consequence of accidental traumatic excitation of the periosteum or nerve fibers in the spinal canal. This phenomenon may be elicited by a control puncture without the injection of any anesthetic, although it is not consistently reproducible even by a painful stimulus. Where such reduction in clearances occurs, the disturbance is fleeting (10 to 20 minutes), and in the data presented here we have excluded the first 20 to 30 minutes after puncture if any obvious disturbance did occur.

Renal blood flow From previously published observations (18) and from unpublished data it can be stated that the blood flow through the normal human kidney under essentially constant arterial pressure can vary through a wide range, the extreme values observed by us to date in normal individuals being about 600 to 3000 cc per minute. However, the renal blood flow tends to remain quite constant on any one day or on weekly re-examinations, unless it is altered by factors obviously of such a nature as to modify the renal circulation (adrenin and other drugs, pyrogenic reaction, etc.). When changes in the renal blood flow are so induced they are accompanied by changes in the filtration fraction, indicating a disturbance of the relative tonus of the afferent and efferent glomerular arterioles. The extreme values of the filtration fraction that we have observed under various circulatory conditions are about 0.08 and 0.33.

From our present data on the effects of spinal

anesthesia on renal blood flow we present in detail two series of observations in Figures 1 and 2 which are fully explained in the legends and a summary of pertinent data on all subjects in Table I (Six subjects in whom no clearances were determined during the pre-anesthesia control period are omitted from Table I, but this table includes 2 subjects who are not included in Figures 3 and 4, in whom anesthesia rose only to T6 and

T9 (Numbers 19 and 20)) Circulatory tests, as described in Section 4, were made upon a number of these subjects both before and during anesthesia, in only a few instances did these tests have any apparent effect on the renal circulation, and in analyzing the data with respect to the effect of anesthesia upon renal blood flow we have with few exceptions compared the average of three clearance periods before anesthesia with the average

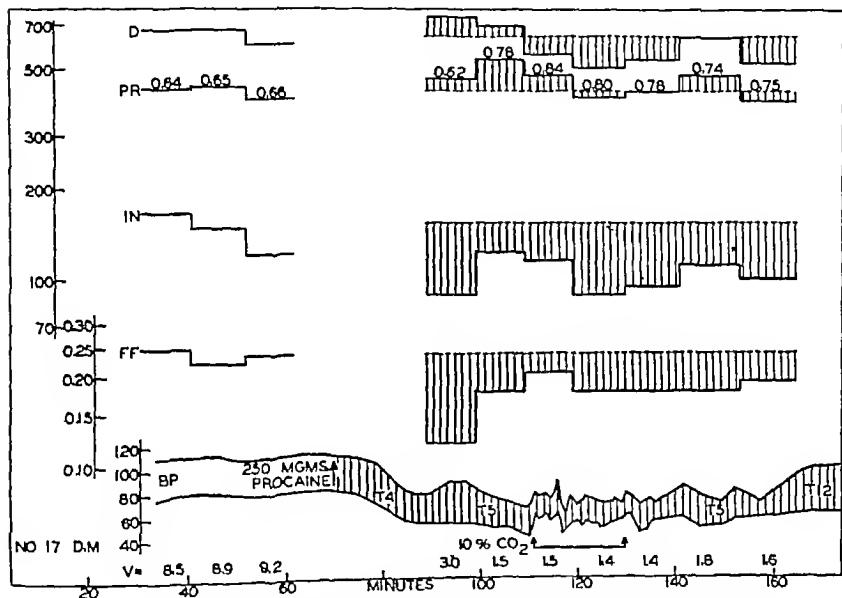


FIG. 1 THE EFFECT OF SPINAL ANESTHESIA ON RENAL FUNCTION AND ARTERIAL PRESSURE IN SUBJECT 17

During the period indicated 10 per cent CO_2 in air was administered by an anesthetic face mask. The data from above downward are

D Diodrast plasma clearance = effective renal plasma flow in cc. per minute
PR Phenol red plasma clearance in cc. per minute. The figures above this curve indicate the PR/D clearance ratio
IN Inulin plasma clearance = rate of glomerular filtration in cc. of plasma per minute.
FF Filtration fraction (i.e. fraction of plasma filtered through the glomeruli) = IN/D
BP Auscultatory brachial pressure in mm. Hg The figures within the *BP* curve show the spinal level of complete sensory anesthesia.

V Urine volume in cc. per minute.

This subject showed 8 per cent decrease in the mean renal plasma flow during anesthesia up to T5 as compared with the 3 control periods before anesthesia. The marked drop in the absolute filtration rate (33 per cent) and in the filtration fraction (27 per cent) is probably attributable to the fall in arterial pressure, the mean value of which decreased 33 per cent in consequence of anesthesia. This is the most marked fall in filtration rate observed, and should be compared with Figure 2 or the data in Table I

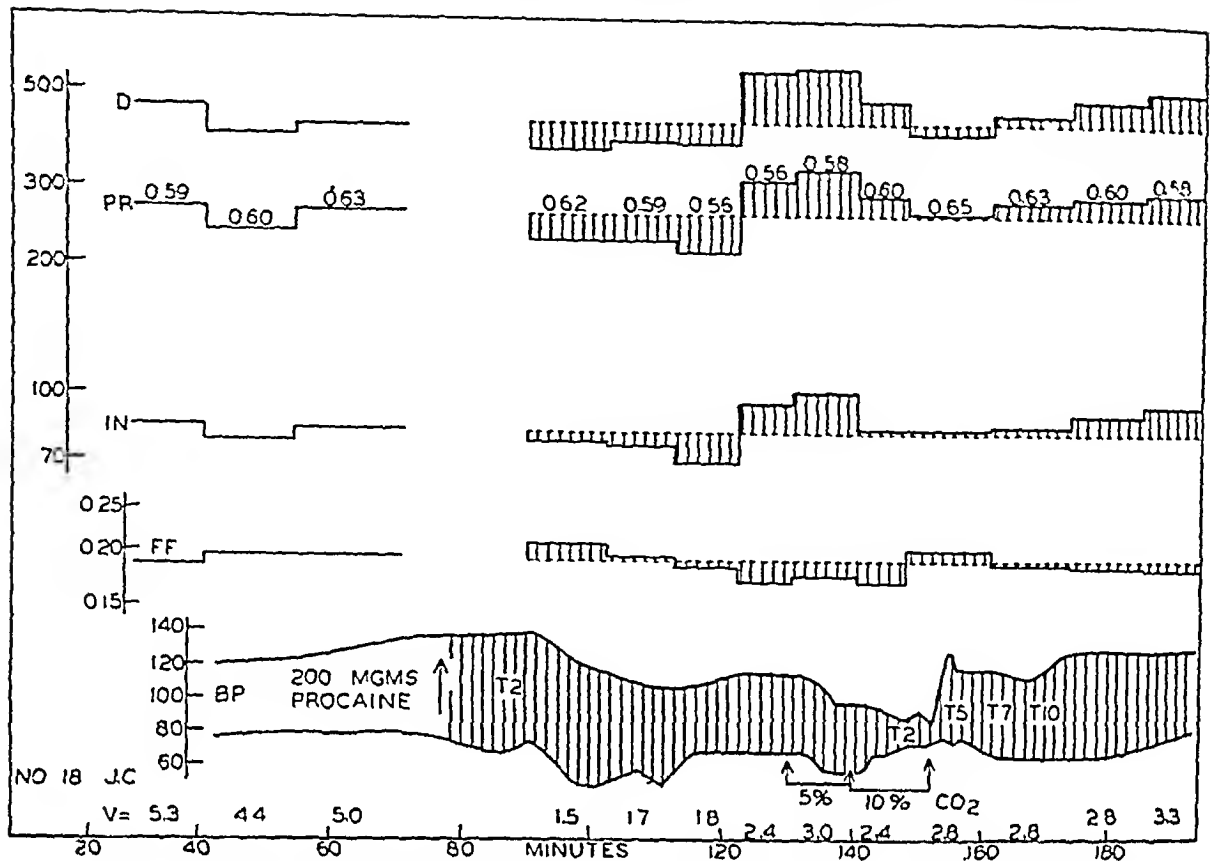


FIG. 2. THE EFFECT OF SPINAL ANESTHESIA ON RENAL FUNCTION AND ARTERIAL PRESSURE IN SUBJECT 18

See Figure 1 for meaning of data. This subject showed an increase of 8 per cent in renal plasma flow and a 4 per cent decrease in filtration fraction during anesthesia up to T2. This response resembles the majority of those listed in Table I.

of all clearance periods while anesthesia was at its height.

If vasotonic impulses, necessary for maintenance of either afferent or efferent arteriolar tone, are carried to the kidneys by the sympathetic nerves, anesthetization of the spinal roots up to T5 or higher would be expected to result in changes of considerable magnitude both in the renal blood flow and in the filtration fraction. So long as the arterial pressure remains constant these changes would, in theory, be of the following qualitative nature: (a) Afferent dilatation alone would be accompanied by an increase in both the renal blood flow and the filtration fraction, the latter rising in consequence of increased glomerular pressure. (b) Efferent dilatation alone would be accompanied by an increase in the renal blood flow, but a decrease in the filtration fraction, the latter falling in consequence of a decrease in

glomerular pressure. (c) Simultaneous afferent and efferent dilation should be accompanied by the greatest increase in renal blood flow without marked changes in the filtration fraction. (d) Apart from the foregoing factors, any change in arterial pressure would tend to change both the renal blood flow and the filtration fraction in the same direction.¹

Applying these general propositions to the first

¹ If equilibrium between the glomerular filtration pressure and capsular pressure is not normally reached before the blood emerges from the glomeruli, any change in the renal blood flow would itself alter the filtration fraction by prolonging the time of contact. The effects of variation in the time of contact and of filtration pressure would be in the same direction in consequence of changes in efferent arteriolar tone, and in the opposite direction in consequence of changes in afferent arteriolar tone. These two factors cannot be definitely separated at the present time.

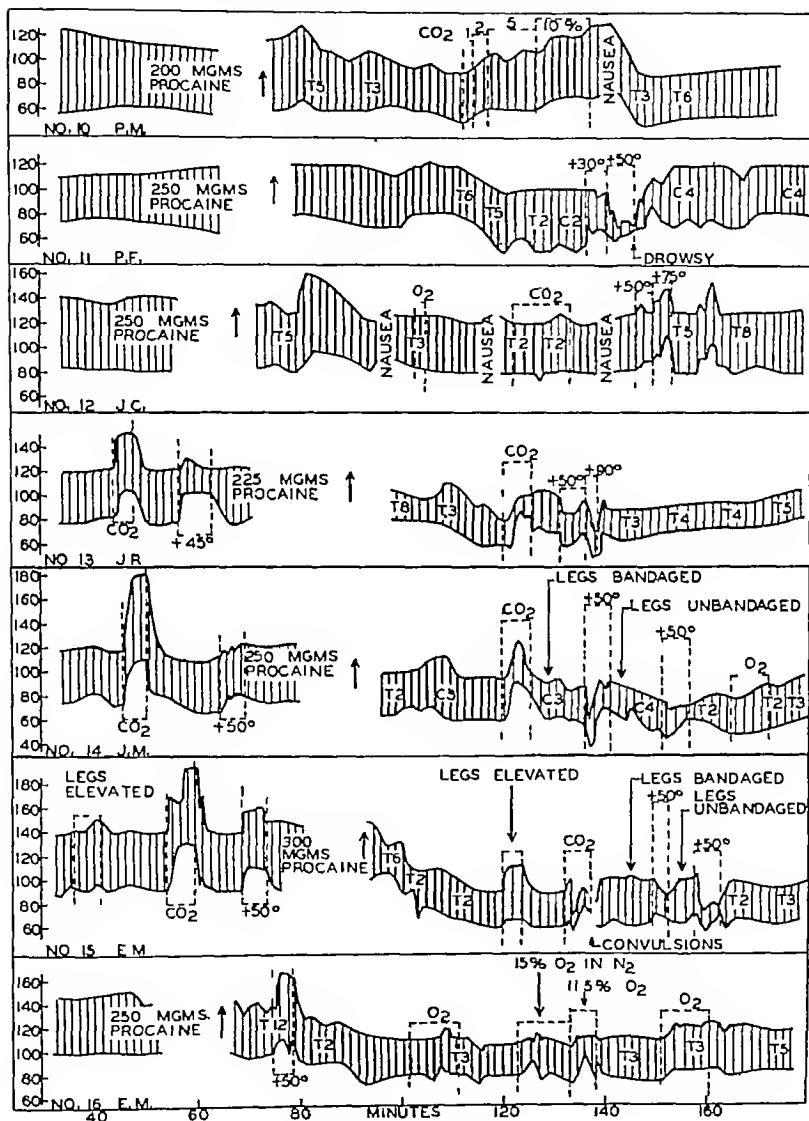


FIG. 4 Subjects 10 to 16

Legend as in paragraph 1 under Figure 3

in both of whom anesthesia extended up to T₂, the systolic and diastolic pressures were maintained at levels equal or superior to the pre-anesthesia control period (Note that in Number 2, the systolic pressure fell only as the anesthesia receded). In the remaining 16 subjects there was some decrease in either the systolic or diastolic pressure, or both. The systolic pressure fell more than the diastolic in every instance except Number 18. In all but 5 instances (Numbers 4, 5, 8, 11, and 17) the diastolic pressure was maintained at 60 mm Hg or above, and frequently it did not fall below its control value (Numbers 1, 2, 3, 10, and 12). The changes in systolic and diastolic pressures are not suited to summary description, but the average pressures are tabulated in Table I.

The type of blood pressure response observed here is, from the point of view of hemodynamics, not consonant with arteriolar dilatation. The fact that the diastolic pressure may not fall at all, that it rarely falls below 60 mm, and that it invariably falls less than the systolic pressure, is directly opposed to the changes to be expected during arteriolar dilatation. Excluding for the moment any volume changes in the post-arteriolar bed, arteriolar dilatation must in principle reduce the peripheral resistance, and therefore, at a constant cardiac output, it will reduce the diastolic pressure, as arteriolar dilatation progresses the diastolic will fall to proportionally lower levels until stagnation in the enlarged arterioles precipitates peripheral circulatory failure. Moderate arteriolar dilatation, by increasing the vascular volume, will also lower the systolic pressure, but not to the same degree as the diastolic, and it therefore will reveal itself in its moderate stages by a substantial increase in pulse pressure at the expense of the diastolic pressure. Insofar as increased capillary volume results from arteriolar dilatation it can only affect this picture by exaggerating these changes. Opposed to the tendency of arteriolar dilatation to lower the systolic pressure will be any increase in cardiac output which may result from increased venous pressure, and it is conceivable that with moderate dilatation this pressor factor could nearly balance the depressor factors, so that the systolic pressure would be well maintained.^{*}

^{*} Perhaps the best examples of arteriolar dilatation are afforded by the action of adrenin and ephedrine,

The responses recorded in Figures 3 and 4 are rather those to be expected where the peripheral resistance has remained unchanged, but where the cardiac output has been reduced. During spinal anesthesia the heart rate typically remains constant (60 to 70) in unmedicated subjects, at a slightly lower rate than during the control period (70 to 80), other factors remaining equal, increased stroke volume would probably prevent any decrease in cardiac output, and, to account for the reduction in arterial pressure we are forced to look for a decreased stroke volume occasioned by a decreased venous pressure. The most likely factors tending to decrease venous pressure in these subjects are dilatation of capillaries, venules, and veins in the skeletal muscles, not in consequence of the abolition of vasotonic impulses, but simply in consequence of the loss of skeletal muscle tone. It may be that additional venous stagnation occurs in the abdomen in consequence of paralysis of the abdominal muscles, and also in consequence of the loss of the reciprocal tonic relationship between the abdominal and thoracic muscles, though the contribution of these factors is uncertain. That the skin arterioles are dilated during spinal anesthesia is reliably indicated by the fact that the skin temperature usually rises, and by plethysomographic measurements (see Section 3), although this dilatation would of course contribute to the expansion of the vascular bed, it would also tend to elevate venous pressure and offset such venous stagnation as may have occurred.

which lower the peripheral resistance and raise the systolic pressure by increasing cardiac output, chiefly through increasing the venous pressure (the constriction of the skin arterioles and perhaps the veins compensating for the enlarged vascular bed in dilated areas). For illustrations of the action of adrenin or ephedrine on blood pressure in man the reader is referred to the latter part of Number 4 in Figure 3 of this paper, or to Figure 6 of Chasis *et al.* (18), and to the measurements of cardiac output and peripheral resistance by Starr, Gamble, Margolies, Donal, Joseph, and Eagle (97), Böger, Deppe, and Wezler (8), Wezler and Böger (105a) and Meyer and Spiegelhoff (67a).

* CoTui (20) has shown that during spinal anesthesia in the dog the spleen may expand by an amount equal to one per cent of the body weight, or roughly 12.5 per cent of the blood volume, while Barcroft and Elliott (5) report that the splenic volume may change by an amount in excess of 10 per cent of the blood volume. Some splenic dilatation in man during spinal anesthesia appears

Relatively moderate hemorrhage cannot be tolerated by an animal in which compensatory vasoconstriction is prevented by sympathectomy or spinal anesthesia a loss of 15 per cent of the blood volume may reduce the blood pressure to critically low levels, and a loss of 20 per cent may be lethal (82, 14, 56). Expansion of the capillaries, venules and veins of the flaccid muscles and of the abdominal veins would be equivalent in a subject with vasomotor paralysis below T5 to a hemorrhage of this magnitude with no arteriolar dilatation other than in the skin to offset this stagnation by raising venous pressure, the cardiac output would unquestionably be reduced sufficiently to lower the arterial pressure to the extent observed. The reduction of cardiac output need be of no great magnitude, since vasoconstriction in the arms and head could offer but negligible compensation in maintaining the arterial pressure in the face of the decreased cardiac output.

We suggest therefore, that the fall in blood pressure in spinal anesthesia results not from arteriolar dilatation, but from a reduction in cardiac output in consequence of venous stagnation. This is essentially the interpretation offered by Gray in 1909 (37) and by Gray and Parsons in 1912 (38) who were the first to consider this problem critically, and for our emphasis upon the venous side of the circulation a precedent has long been established by Yandell Henderson (45). As will be shown later there is little evidence that arteriolar dilatation occurs and much evidence that it does not.

4 *The effects of anesthesia on the reflex responses to posture hypercapnia and anoxia*

Posture Since it is known that sensory fibers are more readily blocked by local anesthetics than are motor fibers (33) it was necessary to inquire whether the vasomotor fibers in the anterior roots of our subjects were actually anesthetized. To obtain information on this point we utilized the reflex responses to posture and CO_2 .

to be the rule, though precise information on this point is lacking. Although hemodynamically the spleen may play a rôle comparable to a major arteriovenous fistula (60) the possible importance of its dilatation in increasing the volume of the post-arteriolar bed cannot be overlooked.

When the normal subject assumes the upright posture, reflex vasoconstriction mediated through the aortic arch-carotid sinus receptors and the bulbar reflex centers serves to maintain the arterial pressure with but little change. (See control observations on Numbers 13, 14 and 15.) In our subjects with spinal anesthesia, bending the body at the waist to an angle of 50° had little effect on the arterial pressure (Numbers 12 and 13) while an angle of 75° produced a slight rise in pressure in Number 12 and an angle of 90° produced a fall in pressure in Number 13. In Number 11 the diastolic pressure was maintained but the pulse pressure thinned out in the manner characteristic of a failing venous return. Only in one instance (Number 9) did slight elevation (20°) lead to syncope,* but it is probable that none of our subjects could have tolerated the upright position for long without syncope, for progressive failure of the circulation would most certainly have occurred. We endeavored to determine the effect on the blood pressure of tightly bandaging the legs with an Esmarch bandage during tilting (Numbers 14 and 15) but the results were indeterminate apparently as much stagnation occurring in the abdomen as in the legs.

The above observations demonstrate that the characteristic responses to gravity namely, a rise in diastolic pressure and a well maintained systolic pressure, were absent in our subjects indicating that the vasomotor pathways were effectively blocked.⁵ The observed responses are not such as are to be expected if there is maximal dilatation of the vascular bed, in which circumstance immediate syncope should almost certainly occur. On the contrary, they suggest moderate capillary-venous stagnation, aggravated by gravity.

Hypercapnia In normal animals and man the inhalation of gas mixtures containing CO_2 produces an abrupt rise in both systolic and diastolic pressure, this reflex response being mediated in part through the carotid receptors and in part

* Interestingly this was the subject who showed renal hyperemia (see Table I).

⁵ What appears to be a reflex response to tilting was obtained in Number 16, but here the level of anesthesia was only up to T12 at the time of tilting although we may presume that the legs could not participate in this response, the major splanchnic vasomotor paths were still intact.

through the bulbar centers. Ten per cent CO_2 is sufficient to produce a maximal effect (42, 57, 59, 64, and 75). Our gas mixtures were prepared by using an anesthetic appliance equipped with accurate metric flow meters and were not analyzed in every case, but from the analyses of some of them we can state that they ranged from 10 to 14 per cent. In Numbers 13, 14, and 15 the subject was tested with CO_2 immediately before anesthesia to demonstrate that he gave the typical normal response. Two features in this normal response are to be noted: the rise in blood pressure occurs immediately, and the pressure remains at a maximal value so long as hypercapnia persists. As demonstrating the response of subjects with high spinal anesthesia to hypercapnia we call attention to Numbers 10, 12, 13, 14, and 15 in Figure 4, and to 17 and 18 in Figures 1 and 2. CO_2 may sometimes cause a rise in pressure, but the rise is slow and progressive (Number 10) or slow and transient, disappearing during the hypercapnia (Number 14). More frequently the response consists of a distortion of the pulse pressure without a significant rise in mean pressure (Numbers 13, 15, 17, and 18). In one subject (Number 12) 10 per cent CO_2 had absolutely no effect on the blood pressure.

It is to be emphasized that these high concentrations of CO_2 produce an extreme hyperpnea which must profoundly influence cardiac filling; it is to be expected that the precise effect in any instance will be influenced by the degree of synchronization of the diaphragmatic movements (the intercostal muscles being paralyzed) with the slow heart rate (60 to 70) obtaining in these subjects. Further factors influencing the contour of the pulse pressure under CO_2 are the possible vasoconstriction in the unanesthetized head, arms, and hands, mediated through the upper thoracic segments, and the pressure of the diaphragm upon the abdominal viscera, in consequence of its extreme excursions. Moreover, there may be small quantities of sympathin E thrown into the general circulation from such upper thoracic sympathetic fibers as are still intact, and this hormone may

cause slight vasoconstriction elsewhere in the body. The variety of changes in blood pressure which we observe under CO_2 we would attribute to these causes, and chiefly to the effect of the hyperpnea on cardiac filling. In no case does the character of the blood pressure response to CO_2 in the anesthetized subject reproduce the normal reflex response. We believe, therefore, that the CO_2 test demonstrates that under the conditions of our observations (anesthetization to T5 or higher with the subject initially in the face down position) the vasomotor pathways emerging below T5 are effectively blocked.

Anoxemia. We have produced anoxemia in three subjects, in Number 7, when anesthesia was at the level of the neck (cervical cord) a $\text{N}_2\text{-O}_2$ mixture containing 86 per cent O_2 was administered by a small anesthetic face mask for 18 minutes; there were slight variations in pulse rate, and this remained at about 70 until the end of the anoxic period. After 11 minutes of anoxia, the subject became apprehensive and began to perspire; at 17 minutes twitching movements appeared about the eyes, and the fingers showed spasm; there was profuse perspiration of palms and face, but no perspiration on the body below the clavicle (i.e., in the anesthetized regions); premature contractions appeared, and the subject complained of dizziness and became lethargic. Consciousness was not lost and removal of the mask effected prompt relief from the anoxic symptoms. A sample of arterial blood taken just before the end of the anoxic period showed 18.1 volumes per cent O_2 capacity, and 8.6 volumes per cent O_2 content, or 48 per cent saturation. The systolic pressure in this subject fell, but the diastolic remained unchanged. In Number 16, a $\text{N}_2\text{-O}_2$ mixture containing about 15 per cent O_2 was administered for 8 minutes, after which the O_2 content was decreased to 11.5 per cent, this mixture being administered for an additional 5 minutes. No signs of anoxia appeared with the first gas mixture; after 2 minutes with the second mixture cyanosis was evident and premature contractions appeared. The heart rate increased momentarily from 70 to 78, but was at 70 at the conclusion of the anoxic period. Arterial blood taken at the end of the anoxic period showed 60 per cent saturation, in comparison with 84 per cent saturation in a sample taken just before the anoxic

* We fully realize that the brachial pressures as measured by auscultation are not accurate measures of the intra-arterial pressures, but the qualitative changes and mean value unquestionably follow the true values closely enough to warrant the conclusions drawn here.

period (The low value of the first sample was unquestionably due to thoracic muscle paralysis.) Again the blood pressure showed no marked changes. In Number 8, a gas mixture containing 8.6 per cent O_2 in N_2 was administered for 4.5 minutes. After 1 minute the subject became apprehensive and restless in 2 minutes twitching movements appeared about the eyes and the fingers showed spasm, after rapidly developing cyanosis the subject began to yawn and grit his teeth and he became drowsy and went into syncope, pure O_2 was given under pressure for 2 minutes during which time the cyanosis cleared and consciousness was recovered. Arterial blood taken before anesthesia showed 12.9 volumes per cent O_2 capacity, 12.6 volumes per cent O_2 content or 96 per cent saturation; a second arterial blood sample taken during the period of anoxia and just before syncope showed 13.6 volumes per cent O_2 capacity, 5.5 volumes per cent O_2 content or 42 per cent saturation. The response in this subject was complicated by the initially low O_2 capacity which was attributable to anemia. The abrupt nature of the syncope suggests that it was of the vasovagal type, precipitated by cerebral anoxia, but in any case we do not believe that it can be accepted as indicative of the normal response, especially in view of the almost equally severe anoxemia induced in the other two subjects. We did not produce anoxemia in our subjects during control periods but from the fact that O_2 lack is normally accompanied by a rise in blood pressure, our failure to obtain this effect during anesthesia affirms our conclusion that the vasomotor paths were actually blocked. However, the chief value of our observations on anoxia will be brought out in Section 8.

PART II

Discussion

If the interpretation given above is correct, it involves the essentially complete abandonment of the view that vasomotor impulses from the central nervous system are necessary in a subject at rest to maintain the peripheral arteriolar bed in its normal tonic state, not only in the kidneys but also in the viscera and possibly in the skeletal muscles. In this interpretation we are at variance, not only with nearly all investigators of spinal an-

esthesia who have tacitly or explicitly accepted dilatation of the arterioles of the splanchnic and other regions as the major hypotensive factor (10, 12, 13, 20, 27, 56, 62, 67, 81, 88, 89, 94 and 98), but also with the conclusions drawn from numerous animal experiments upon which the accepted idea of the tonic activity of the sympathetic nervous system is founded. In view of this fact it is necessary to examine the contrary evidence critically.

5 The alleged tonic activity of the vasoconstrictor paths

The notion of vasotonic activity in the sympathetic nervous system in the normal animal is based largely upon the fact that the blood pressure falls or the peripheral blood flow increases immediately after surgical section of the splanchnic fibers in anesthetized cats and dogs. It must be noted, however, that surgical section of the splanchnic nerves, the lateral ganglia, the anterior roots of the cord or the cord itself involves traumatic excitation not only of vasoconstrictor fibers but also of the vasodilator fibers to the viscera, skin, and skeletal muscles. But what is more important is the fact that such observations have invariably been made in anesthetized animals.

In 1912 Elliott (23) showed that during anesthesia with ether, chloroform, or urethane an intact adrenal gland in the cat may be nearly depleted of its content of adrenin whereas a denervated gland in the same animal is unaffected. Cattell (17) demonstrated in 1923 that ether anesthesia increased the perfusion pressure and caused a diminution in the volume of a normal limb, but not in a denervated limb, which phenomenon he attributed to excitation of the vasomotor center. More recently, Bhatia and Burn (7) have shown that ether excites both the central and spinal sympathetic centers. Ether also causes constriction of the spleen (44). Evidences of sympathetic excitation in man during ether anesthesia have been listed by Knoefel (61) and so marked is the systemic pressor action of ether that its inhalation or subcutaneous injection has actually been used to elevate the blood pressure during spinal anesthesia (98, 67, 1). On the other hand, ether anesthesia is accompanied by hyperemia in the hind limb (55) by a decreased O_2 A-V difference (right heart blood) in the

dog (90), by elevation of the skin temperature in man (21), and by other evidences of vasodilatation. Consequently, the overall effect of ether anesthesia is difficult to evaluate since the sympathetic nervous system carries dilator fibers to the viscera as well as to the muscles in the dog, and perhaps also in man. Possibly, as Burn (15) points out, it excites the "vasodilator center" as well as the vasoconstrictor center. It is clear that during ether anesthesia either vasoconstriction or vasodilatation may predominate,⁸ as White (106, p. 121) suggests, splanchnic constriction may occur simultaneously with skin dilatation.

An equally severe arraignment can be levelled against observations made on morphinized animals. Morphine, in addition to slowing the heart by vagal action, depresses the respiratory center and consequently promotes anoxia and hypercapnia (76). It is reported that in sympathectomized cats and dogs the blood pressure is quickly lowered to critical levels by slight degrees of either hypercapnia or anoxia (see Section 4), and it is conceivable that these factors contribute to the marked hypotension which accompanies surgical denervation or spinal anesthesia in morphinized-etherized animals. Furthermore, morphine (and more especially scopolamine) causes relaxation of the skeletal muscles and thus promotes venous stagnation. That the hypotension of spinal anesthesia in dogs is in fact aggravated by morphine, scopolamine, barbiturates, and other anesthetics has been pointed out by Seevers and Waters (89).

In view of the above facts, we may confidently reject the acute effects of denervation on blood pressure or vascular tone, when effected under ether or morphine anesthesia, as having little bearing on the normal animal. In the absence of more information this same criticism must be applied to barbitol, amytal, *etc.*, which cause marked dilatation of the spleen (44) and modify many autonomic responses (15), and to urethane, which is known to evoke vasoconstrictor activity (7).

Even excluding the possible effects of the surgical excitation of vasodilator fibers, and of anes-

thetics, there remains the question to what extent the delicate pattern of the vasomotor system and the set of the presumptively autonomous peripheral vasomotor apparatus are caused to depart from normal by the procedures of opening the abdomen and fingering the viscera whereby they may suffer some local excitation, if not traumatic injury, by handling, drying, and chilling.

The sympathetic paths are predominantly vasoconstrictor to the skin, that these dermal paths are tonically active at ordinary temperatures (an activity obviously subserving the regulation of body temperature) is indicated by the facts that the temperature of the skin and particularly of the digits in man and of the foot pads in the dog, and the blood flow in the ear of the rabbit (all regions obviously important in body temperature regulation) are increased by sympathectomy (20, 69, 106, 107). But the increased volume of the hand after anesthetic nerve block (30) is scarcely more than might be accounted for by dilatation of the skin arterioles (36) and does not certainly indicate dilatation in the muscles, much less in the viscera.

Spinal anesthesia is reported to increase the rate of flow of perfusion fluid through the femoral and brachial arteries of the dog (13), but these observations are complicated by barbitol anesthesia. The most creditable evidence of denervation dilatation in the skeletal muscles is the fact that lumbar sympathectomy produces a permanently increased blood flow in the femoral artery of otherwise normal dogs (thermostromuhr method under local anesthetic (54, 55)), and the increased flow is larger than probably can be accounted for by dilatation of the vessels of the skin and paw. But there remains the question of whether sympathetic activity is not evoked *de novo* or considerably exaggerated in conscious but operated dogs upon handling in the laboratory. If so, the control blood flows may be abnormally low. Moreover, the lumbar sympathetic fibers to the muscles of the dog appear to be predominantly vasodilator, as in man (26, 36, 15) and consequently it would not be expected that transection would result in chronic hyperemia. A re-examination with attention to these questions is needed.

Bradshaw (10) has shown that barbitolized cats with the carotid artery cannulated show a marked fall in blood pressure during spinal anes-

⁸ In anesthetized animals small doses of adrenin cause a fall in arterial pressure, a phenomenon not observed in unanesthetized animals (22) or man, and many other physiological inversions under anesthesia could be listed if space permitted. Adrenin may also have a hypotensive action in the sympathectomized dog (105).

thetia, whereas sympathectomized cats subjected to the same procedure ten days after operation showed no change in blood pressure. He concluded that the fall in blood pressure in the control cats was due entirely to anesthesia of the vasoconstrictor fibers. But the fact that barbital, in addition to modifying other sympathetic responses dilates the spleen (44) and relaxes the skeletal muscles, and may conceivably promote anoxia or hypercapnia, casts doubt upon these observations. In the sympathectomized cat no compensation (by sympathetic excitation) could be effected for venous dilatation induced by barbital, as in the controls.

Shaw, Steele, and Lamb (91) have worked with dogs which received only local anesthesia for cannulation of the vena cava and femoral artery, and fleeting general anesthesia with ethyl chloride during the intrathecal injection of large doses (10 to 20 mgm. per kgm.) of procaine. Though these investigators were not concerned with the present issues, their experiments are unimpressive from the physiological point of view. After spinal anesthesia in one dog the blood pressure remained *unchanged* in another it *increased* while in five others, during the first 30 minutes it fell by an average of 37.5 per cent of the control value. These investigators find (as had been demonstrated by CoTui (20)) that the O_2 A-V difference (right heart blood) is invariably increased (because of a fall in the O_2 content of venous blood) a result entirely opposite to what occurs in ether anesthesia (90). Under the presupposition that vasodilatation is the cause of the fall in blood pressure, Shaw and his coworkers were forced, in order to explain the paradoxically increased O_2 A-V difference, to assume that arteriolar dilatation had caused the blood to stagnate in the anesthetized regions. Their data are open to another interpretation: the increased O_2 A-V difference could well be the result of a decreased cardiac output and in fact, the parenthetical mention by these investigators of the existence in their dogs of pulmonary ischemia and of decreased circulation time support this explanation.

If vasodilatation were the cause of the hypotension of spinal anesthesia it should when the arterial pressure is only moderately reduced be accompanied by an increase in venous pressure and therefore in the stroke volume of the heart.

On the other hand, if venous stagnation is the cause, it should be accompanied by a decreased stroke volume and by a decreased cardiac output.

In dogs Seevers and Waters (89) (barbital) report that the venous pressure is increased by spinal anesthesia but only one animal is cited in detail and in this the venous pressure did not increase until the arterial pressure had fallen from 150 to 90 mm. Bower, Clark, Wagoner and Burns (9) (ether) report no fall in the pressure in the superior vena cava although the pressure in the inferior vena cava is said to have been decreased. Again in dogs Burch and Harrison (12) (morphine and barbital) report that an average decrease in blood pressure of 44 per cent is accompanied by an average decrease in cardiac output of 23 per cent. These authors state that the cardiac output is decreased before the blood pressure, but the evidence presented consists of 2 single measurements. In man, Polano (74) reports no change in cardiac output (Broemser's method) in 5 subjects with spinal anesthesia, and a decrease in 2 subjects, but in no case was there a marked drop in blood pressure. In contrast to these results, Schuberth (86) reports cardiac output in 14 subjects (Grollman's method), some of whom had anesthesia as high as the xiphoid or nipples (All had received 50 mgm. of ephedrin before anesthesia.) In 4 subjects the cardiac output increased in 2 of these the systolic pressure increased, in one it remained unchanged and in the fourth it fell from 155 to 130 mm. But in the remaining 10 subjects the cardiac output *fell* the most marked reduction being associated with the *greatest fall in pressure*. In all but 3 instances the stroke volume decreased. Schuberth notes the collapsed veins as a sign of reduced venous pressure. He also reports a consistent fall in cardiac output in rabbits during spinal anesthesia. The venous pressure (auricular) in rabbits was either unchanged or fell, it never increased except under artificial respiration. In both man and rabbits the O_2 A-V difference was consistently increased. Schuberth concluded that the fall in arterial pressure was a consequence of decreased cardiac output following "an impaired return to the heart" but he attributed this impaired return to paralysis of the vasoconstrictor nerves and does not mention the possibility of pure venous stagnation. Neither he nor Shaw

Steele, and Lamb (91) discuss the difficulties of reconciling *decreased* venous pressure, *decreased* cardiac output, and *increased* O_2 A-V difference, with the notion of arteriolar dilatation, which, short of circulatory collapse, should tend to *increase* venous pressure and cardiac output and *decrease* the O_2 A-V difference. Schubert's control observations did not immediately precede the observations under anesthesia, yet the absolute figures for the cardiac output are so low in a large percentage of the subjects examined by him as to leave no doubt as to their qualitative significance. Accrediting such measurements⁹ after partial or complete thoracic paralysis, a reduced cardiac output, sometimes to a very low level, can be accepted as a fact. This fact, then, combined with the blood pressure picture described here, points conclusively to venous dilatation, rather than arteriolar dilatation, as the cause of the fall in arterial pressure.

6 The evidence bearing on the autonomy of the peripheral arteriolar bed

In explaining the hypotension of spinal anesthesia, it may be noted that Nowak (70) has argued in favor of a toxic effect of the anesthetic after systemic absorption, but this explanation seems to have been excluded by the intravenous injection of large doses (27, 9). We have observed no evidences of systemic toxicity or central respiratory depression in spite of relatively large doses of procaine intrathecally.

Taking first the evidence obtained by surgical denervation, it has long been known that after spinal transection the blood pressure tends to return to normal levels, the restoration possibly being due, in part, to subsidiary vasomotor centers in the cord (66, 58). But the denervated vessels of the ear in the rabbit, of the paws in dogs, *etc.*, and of the hands and feet in man, ultimately recover their normal caliber, demonstrating that even in the skin, where tonic vasomotor activity

must be accepted, the vascular bed possesses at least a latent capacity for autonomous constriction (66, 32, 106, 107).

The recent observations of Cannon and of Hermanns and their respective collaborators (2, 3, 4, 16, 73, 79, 82) have shown that the peripheral vasomotor apparatus can re-establish an essentially normal blood pressure after complete sympathectomy, which appears to destroy all constrictor paths to the splanchnic viscera, skin, and muscles only the dorsal root dilators and the vagi remaining intact (101, 79, 68). Rowntree and Adson (80) have reported a subject with polyarthritis in whom the blood pressure returned to normal after cervicothoracic and abdominal ganglionectomy. In all these instances, however, a considerable interval was allowed to elapse after denervation before pressure readings were taken, affording an opportunity for the peripheral vasomotor apparatus to acquire slowly and *de novo* a degree of autonomous activity perhaps not present in the normal animal. It is, in fact, commonly believed that such is the actual course of events, a belief which rests chiefly upon the observed restoration of arteriolar tone in the denervated ear of the rabbit and the denervated skin in dogs or man.

But against this belief may be presented the evidence afforded by the experiments of Hermann, Morin, and Vial (46, 48, 49), who have destroyed the thoracic, lumbar, and sacral cord by a technique which permits quick restoration of blood pressure. The pressure is elevated, presumably by vasoconstrictor excitation, at the moment of trauma of the cord, thereafter falling to 70 to 80 mm Hg, but it recovers to 110 to 140 mm shortly after the anesthetic has worn off. After vagotomy these "cervical" dogs show no reflex responses to excitation of the central end of the depressor nerve, the bulb or cervical cord, and it is concluded that no vasomotor connections pass to the periphery except from the spinal cord below T1 (49). Here the restitution of blood pressure might be attributed to the autonomy of the lateral and collateral ganglia, though Hermann, Morin, and Cier (52) adduce evidence that these ganglia play a negligible rôle. In any case, these investigators have fully demonstrated that a pressure of 100 to 140 mm can be maintained permanently in the dog from a few hours onwards after all con-

⁹ Neither Seevers and Waters (89) nor Bower *et al* (9) discuss the significance of venous pressure measurements relative to the atmosphere before and after paralysis of the thorax, nor consider the effect of position of the animal, which presumably was tied down upon its back. And neither Polano (74) nor Schubert (86) question the validity of cardiac output measurements by indirect methods during thoracic paralysis.

nections with the central nervous system have been destroyed up to T1

The most pertinent observations on blood pressure immediately after sympathectomy are those of Grimson, Wilson, and Phenuster (40), whose blood pressure readings were taken on unanesthetized dogs 3 days after the last stage of denervation. They report that the blood pressure, which averaged 155 mm before operation, was lowered by an average of 38 mm. by denervation, there was no decrease in blood volume to explain the lowered blood pressure, nor was there any increase in volume such as might be expected in the event of an appreciable dilatation of the vascular bed. The pulse rate in 3 dogs was slowed by the operation from 109 to 69, and the minute cardiac output estimated twice before and twice soon after operation in 4 dogs averaged 28 per cent lower postoperatively. The authors say: 'The fact that despite the lowered cardiac output and slight bradycardia a blood pressure averaging 117 mm of mercury was maintained shows that the animals suffered only moderate lowering of peripheral resistance as a result of sympathectomy. This indicates that there is an inherent vascular tone which assists in the maintenance of the blood pressure at a reduced level immediately after sympathectomy. This is a conservative statement for the reduction in pressure after sympathectomy may be only apparent and due entirely to the fact that the blood pressure in the control animals was elevated by excitement. In fact Gregg, Eckstein and Fineberg (39) report that the normal blood pressure of the well trained dog approximates 124/85 mm Hg and Verney and Vogt (105) cite 100 to 120 mm as the range of mean arterial pressure. These figures are substantially lower than the control figures of Grimson *et al.* (40) and are at the level of the figures observed in sympathectomized animals.

The observations of Hermann and his collaborators strongly suggest that the autonomy of the vascular bed in the dog adequately demonstrated in the chronically sympathectomized animal, can carry on within a few hours after denervation. If such is the case, it should be immediately demonstrable after non traumatic denervation by spinal anesthesia.

So far as man is concerned, it is asserted by surgeons that one of the advantages of spinal an

esthesia lies in the circumstance that the viscera show no hyperemia neither the intestines nor the uterus bleeding so freely as during inhalation anesthesia. It was from this fact that Gray (37) and Gray and Parsons (38), who were among the first to inquire critically into this problem, asserted that the fall in blood pressure was caused not by arteriolar dilatation but by decreased venous pressure. The anemic appearance of the viscera has sometimes been attributed to reduced blood pressure but it occurs without appreciable reduction in blood pressure and may equally well be attributed to the contracted state of the viscera supplemented by normal arteriolar tone.

Bower, Clark, Wagoner and Burns (9) were unable to demonstrate any increase in the volume of the leg ileum or kidney in the dog during spinal anesthesia and rejected the possibility of vasodilatation. They alternatively attributed the hypotension to respiratory embarrassment, pulmonary congestion and cardiac failure in consequence of paralysis of the cardiac nerves (?) though the last two factors do not appear to be significant.

It is clear that anesthetic denervation can be effected in the dog without substantial reduction in arterial pressure. We have already referred to the report of Shaw, Steele and Lamb (91) that the blood pressure does not invariably fall, and Lundy (65) reports no change in blood pressure in two dogs which were maintained by artificial respiration and with complete paralysis not only of the cord but also of the cervical and cranial nerves. Seever and Waters (89), having found that anoxemia reduces the blood pressure in the spinal anesthetized dog apart from the mechanical movements of the thorax, utilized O₂ administration to maintain the pressure and cite an instance in which the pressure was raised in this manner from 120 to 200 mm (88). In a later paper they report two dogs having control pressures of 144 and 170 mm., after high spinal anesthesia the pressure was maintained during artificial respiration with a modified Drinker respirator at 150 and 180 mm. although it had previously been reduced to low levels in an interval when there was respiratory paralysis. They state that cord section at T5 or anesthesia up to this level results only in the slight drop of 5 to 10 mm. in systolic pressure, the tendency for hypotension to appear

with anesthesia above this level they attribute to anoxia resulting from intercostal paralysis, an interpretation which has also been offered by Heymans, Bouckaert, and Bert (56). None of the investigators mentioned have taken into account the possibility that tying a cat or dog down upon its back with the legs extended might seriously embarrass the venous circulation. It is difficult to imagine circumstances that would equally embarrass the venous circulation in man.

Consideration of the above evidence leads us to believe that in the dog the peripheral arterioles are capable of maintaining a normal blood pressure, as defined by Gregg, Eckstein, and Fineberg (39) and by Verney and Vogt (105) in trained animals, after either destruction of the cord or rapid non-traumatic anesthetic denervation. Consequently, it is to be inferred that basal arteriolar tone in the dog is not dependent upon the tonic activity of the sympathetic nervous system, and it seems highly probable that this is also true in the cat.

7 Evidence of the autonomous control of the renal vascular bed

Our conclusion that the renal vascular bed is endowed with autonomous activity is supported

by numerous observations on the renal blood flow in the dog, as measured by means of the Rein thermostromuhr. However, with few exceptions these observations have been made on dogs which were anesthetized with ether, morphine, pernocton (sodium butyl- β -bromallyl barbiturate), chloralose or urethane, the abdomen had been opened and the renal artery or vein dissected free and cleaned in order to put the thermostromuhr in place, frequently artificial respiration has had to be used, and a few investigators report that some of their preparations have had to be discarded because of anuria. All these circumstances argue against the physiological significance of the results. It may also be noted that Enger and Gerstner (24) report that a vasopressor principle appears in the blood in consequence of momentary renal ischemia, Enger, Linder, and Sarre (25) report that the blood pressure begins to rise within 1 hour after partial clamping of the renal artery, while Verney and Vogt (105) report a rise in 20 to 30 minutes. Whether this rapidly developing constriction involves the renal circulation itself has not been determined, but it is possible that it does

In many investigations physiological significance has been attributed to changes in renal blood flow observed after some hours of experimentation, the results being equally accredited whether the actual blood flow was 10 or 50 cc per gram of kidney per minute. It is to be inferred that the finer adjustments of the renal circulation are seriously modified if not wholly obscured by the extremely unphysiological conditions under which most of the thermostromuhr observations have been made, nevertheless, these observations all agree in indicating that the renal circulation of the dog enjoys a remarkable degree of autonomy.

The renal blood flow tends to remain unchanged during hemorrhage (77) and during the increased or decreased blood pressure elicited by pressure changes in the carotid sinus or by administration of CO₂ or adrenin (43, 95, 96, 72, 102, 83, 84). The independence of the renal circulation in the face of changing arterial pressure persists after denervation (43, 72). It would seem to be in line with this autonomy that the threshold of the renal vessels for adrenin is much higher than in those vessels in the leg (skin and foot pads?) which are constricted by this hormone (43). Though the kidney does not show "reactive hyperemia" (99), a very significant compensation occurs to renal ischemia when the renal artery is partially closed, the renal blood flow is only momentarily decreased, shortly returning towards normal, and in order to maintain the blood flow at a reduced level the clamp must be repeatedly tightened. This phenomenon has been described independently by Schroeder and Cohn (85) and Enger, Linder, and Sarre (25), and interpreted by them as indicating a local vascular readjustment. The increase in phenol red clearance after clamping the renal artery in the dog, reported by Corcoran and Page (19), is possibly due to this same autonomous dilatation.¹⁰

Schneider and Wildbolz (83) have shown that acute denervation under morphine-pernocton anesthesia leads, after a short period of ischemia, to a slow but marked increase in blood flow (65 to 145 per cent above the control). Herrick, Essex, and Baldes (53) report an even larger increase after denervation under ether anesthesia. On the

¹⁰ Similar autonomous vascular readjustments have been reported in the brain (29).

other hand, Handovsky and Samaan (41), working with conscious dogs (but dogs in which the thermostromuhr was attached to the renal artery and in which the ureters and either the brachial or carotid artery were cannulated), found that splanchnic section under local anesthesia caused a much smaller increase in blood flow (20 to 50 per cent) which, moreover, was transient, lasting only 20 to 50 minutes.

In contrast to the above observations, Rhoads Van Slyke Hiller and Alving (78) measuring the blood flow in an explanted kidney by means of urea A-V difference and the urea clearance found that local anesthesia of the renal nerves in unanesthetized dogs or surgical denervation under ether anesthesia was without consistent effect upon the blood flow. These experiments are open to the criticism that the kidney was already hyperemic, the other kidney having been removed about 2 years previously, and under these conditions it is known that the blood flow through the remaining kidney (dog) is nearly double its normal value (63). We find the renal blood flow in a subject who had had a unilateral nephrectomy 5 years before observation to be nearly as great (1037 cc. per minute) as our average normal figure (1339 cc. per minute). Nearly maximal vascular dilatation might be expected in such a kidney even though its nervous connections are intact. But apart from this criticism the results of Rhoads *et al.* (78) are consonant with our own in indicating no tonic control of the renal circulation in the normal animal.

8 The vasodilating action of hypercapnia and anoxemia on the peripheral vascular bed

The pressor response induced in the anesthetized dog and cat by CO_2 and anoxemia is converted after section of the vasomotor pathways to a profound depressor reaction. The explanation of this phenomenon is not clear. That CO_2 and anoxemia exert a dilating action on arterioles or capillaries in perfused or isolated organs has been repeatedly demonstrated (51), though the application of this evidence to the intact animal is so uncertain that it need not be considered here. But a depressor action is also strikingly evident in intact denervated preparations. The inhalation of gas mixtures containing from 2 to 10 per cent of CO_2 causes a precipitate fall in arterial

pressure in ergotamized dogs (59, 71), in sympathectomized vagotomized cats and dogs (2, 3, 73) in dogs with spinal anesthesia (56, 89, 20, 87) and in cervical dogs with the cord destroyed below T1 (47, 50, 51).

The administration of a gas mixture containing 2 to 4 per cent of CO_2 to Hermann's "cervical" dogs produces a 10 mm drop within 2 minutes, while 10 per cent CO_2 produces a profound drop in an equal time. Anoxemia has a similar effect. 18 per cent O_2 in the respired mixture produces a slight reduction in blood pressure, while 10.5 per cent O_2 produces virtual collapse of blood pressure in 1 to 2 minutes' time. (The normal pressor response to anoxemia is first converted to the depressor response when the cord is destroyed up to and including T7, the depressor response becoming maximal when destruction reaches T3. It appears therefore that the vasomotor pathways necessary for the normal pressor response emerge below T3, and chiefly in T4-5-6 and 7.)¹¹

There appear to be only 3 possible explanations for the depressor phenomenon. (1) CO_2 and anoxia may directly or reflexly cause the secretion of some humoral agent which exerts a dilating action on the vascular apparatus. (2) there may be vasodilator fibers emerging from the cord or brain above C6 which are centrally excited by CO_2 and anoxia. (3) CO_2 and anoxia may exert a dilating action on the peripheral vasomotor apparatus (arteries, capillaries or veins) either by direct action on the vascular tissue or indirectly through peripheral neurons. Since there is no evidence to indicate the secretion of a vasodilating humoral agent, and since there is considerable evidence against the existence of vasodilator paths to the viscera emerging above C6 (49, 79) the direct action of CO_2 and anoxia on the peripheral vasoneural or vascular apparatus appears to be the probable explanation of the fall in pressure. However, if experiments with isolated perfused organs are excluded we are aware of no evidence which will enable us to decide whether this dilating

¹¹ (Note added in proof.) Contrary to the above, Mc Donough (65a) has recently reported that unanesthetized sympathectomized dogs although slightly more sensitive to anoxia than normal dogs, can endure 6 per cent O_2 for 5 hours. This observation illustrates the danger of transferring conclusions from anesthetized to normal animals.

tation involves the arteriolar, capillary, or venous bed

In any case, we are faced with the paradox that in human subjects with spinal anesthesia up to T3 or T2, and in whom all vasomotor reflexes other than those involving the head and arms are demonstrably blocked, neither CO₂ nor anoxemia has any depressor action. We administered CO₂ in a concentration of 10 per cent for as long as 18 minutes (as in Number 17, Figure 1), or (as in Number 15, Figure 4) to the point where involuntary twitching appeared in the unanesthetized forearms and face, without obtaining any depressing effect upon the circulation. (See also Numbers 10, 12, 13, and 14.) Similarly, we are forced to conclude from the responses shown in Numbers 7 and 16 that anoxemia of great severity does not cause dilatation of the peripheral vascular bed in man with high spinal anesthesia. In 3 instances (Numbers 6, 14, and 16) we have administered pure O₂, the rise in systolic pressure in Number 6 might have been due to this measure, but in Numbers 14 and 16 the O₂ had no marked pressor effect. It has been our experience that O₂ relieves the nausea, restlessness, and yawning that signalize bulbar anoxia, but we cannot affirm the inclusion which has been reached in dogs and cats that O₂ raises the arterial pressure. We could not particularly expect such an effect since even severe anoxia does not lower the pressure. It must be noted in this connection that Schubert (86) found no correlation between blood pressure changes and changes in the tidal air of a large series of subjects.

So we are again at variance with the observations on anesthetized dogs and cats, for all of the observations referred to above have been made on anesthetized animals. It may be that the astonishing differences in the innervation of the viscera of the rabbit, hare, cat, dog, and monkey, as described by Burn (15), are presumptive evidence of differences in activity patterns, and, if so, it is clear that studies of the autonomic nervous system and peripheral vasomotor apparatus in other animals must be transferred to man with great caution. That there are species differences in the stability of the peripheral vasomotor apparatus is probable, for the sympathectomized cat suffers an acute fall in arterial pressure and circulatory collapse on slight activity, whereas the

sympathectomized dog is extremely competent physiologically, perhaps even more so than the normal animal (2, 4, 11, 73, 31, 65). But paradoxically, the anesthetized sympathectomized dog is reported to be more sensitive to CO₂ than is the anesthetized sympathectomized cat (4). (See footnote 11 on page 335.)

But anesthesia alters the set of almost every autonomic reflex in the body, it profoundly changes the response of the medullary centers to CO₂ (103,104), and it quite possibly alters the response of the peripheral vasomotor apparatus. Until these observations are repeated on unanesthetized animals, and until consideration is given to the effects of posture on the venous circulation in the cat and dog, it would seem premature to accept as proven that there are any major species differences in the reactivity of the arterioles. If these apparent differences are real, we would be inclined to attribute them to the fact of man's bipedal habitus. We may suppose that the pattern of the vasomotor system, both centrally and peripherally, is adapted to his upright posture and it is rather to be expected that the peripheral vascular bed would in its autonomy show greater stability against vasodilating factors, even of a metabolic nature. This suggestion, however, applies primarily to the arterioles; the capillary-venous circulation, unable to reinforce itself, has remained at a disadvantage, and in man it is possibly more dependent upon skeletal muscle tone and other accessory factors than it is in the quadrupeds.

Our present observations on the importance of dilatation in the post-arteriolar vascular bed in spinal anesthesia add emphasis to the view, long propounded by Yandell Henderson, that the venous side is the weakest portion of the circulatory system.

SUMMARY AND CONCLUSIONS

Twenty-one normal unoperated subjects have been observed before and during spinal anesthesia. In 18 of these subjects sensory anesthesia was established up to T5, and in 3 subjects to above T1. That the anterior roots or sympathetic rami have been effectively blocked has been demonstrated by the abolition of the typical reflex responses to hypercapnia, anoxemia, and gravity.

Anesthesia to levels considerably above those at

41. Handovsky H., and Samaan, A., Observations on the renal circulation and secretion in the dog with special reference to the effect of pituitary (posterior lobe) extract. *J. Physiol.*, 1937 89 14
42. Hardgrove M., Roth, G. M., and Brown, G. E., The pressor reaction produced by inhalation of carbon dioxide studies of patients with normal blood pressure and with hypertension. *Ann. Int. Med.*, 1938, 12, 482.
43. Hartmann, H., Ørskov S. L., and Rem, H., Die Gefäßreaktionen der Niere im Verlaufe allgemeiner Kreislauf Regulationsvorgänge. *Arch. f. d. ges. Physiol.*, 1937 238, 239
44. Hausner E., Essex, H. E., and Mann, F. C., Roentgenologic observations of the spleen of the dog under ether sodium amylal pentobarbital sodium and pentothal sodium anesthesia. *Am. J. Physiol.*, 1938, 121, 387
45. Henderson, Y., Muscle tonus and anesthesia. *Anesth. and Analg.*, 1937 16, 43
46. Hermann, H., Morin, G. and Vial J., Modifications de la pression artérielle au cours et après la destruction progressive de la moelle épinière chez le chien. *Compt. rend. Acad. d. Sc.*, 1934 199, 487
47. Hermann, H., Morin, G., and Vial, J. Démonstration péremptoire de l'action vasodilatatrice périphérique du sang asphyxique. *Compt. rend. Soc. de biol.*, 1934 117 927
48. Hermann, H., Morin, G., and Vial J., Les effets immédiats et lointains de la destruction de la moelle chez le chien. *Compt. rend. Soc. de biol.* 1934 117 967
49. Hermann, H., Morin, G., and Vial J., Evolution de la pression artérielle chez le chien privé de sa moelle dorsale, lombaire et sacrée. *Compt. rend. Soc. de biol.*, 1935 118, 880.
50. Hermann, H., Morin, G., and Vial, J., Influence de l'acapnie et de l'hypercapnie sur les appareils vasomoteurs périphériques. *Compt. rend. Soc. de biol.*, 1935 118 1446.
51. Hermann, H., Morin, G., and Vial, J., Composition gazeuse du sang et appareils vasomoteurs. II. Action sur les appareils périphériques essai de synthèse. *Ann. de Physiol.* 1936, 12, 255
52. Hermann, H., Morin, G., and Cler J., Sur l'activité vaso-tonique des ganglions de la chaîne sympathique. Documents recueillis sur la chien à moelle détruite. *Ann. de Physiol.* 1937 13, 316.
53. Herrick, J. F., Essex, H. E., and Baldes, E. J. Observations on the flow of blood of the kidney. *Am. J. Physiol.* 1932, 99 696.
54. Herrick, J. F., Essex, H. E., and Baldes E. J., Observations on the blood flow in the femoral artery in the dog eight to thirty four months following lumbar sympathectomy. *Proc. Staff Meet., Mayo Clin.*, 1932, 7, 711
55. Herrick, J. F., Essex, H. E., and Baldes E. J., The effect of lumbar sympathectomy on the flow of blood in the femoral artery of the dog. *Am. J. Physiol.*, 1932, 101 213
56. Heymans C., Bouckaert, J. J., and Bert, P., Mécanisme du collapsus circulatoire. Influences sur les réflexes circulatoires sinocarotidiens. *Compt. rend. Soc. de biol.*, 1933 112, 715
57. Heymans C., Bouckaert, J. J., v. Euler U. S., and Dautreban, L., Sinus carotidiens et réflexes vasomoteurs. *Arch. internat. de pharmacodyn. et de therap.*, 1932, 43, 86.
58. Heymans, C., Bouckaert, J. J., Farber S., and Hsu, F. Y., Spinal vasomotor reflexes associated with variations in blood pressure. *Am. J. Physiol.*, 1936 117 619
59. Heymans C., Nowak, S. J. G., and Samaan, A., Sur l'action vasomotrice réflexe, centrale et périphérique de l'acide carbonique, de l'anoxémie et de l'asphyxie. *Compt. rend. Soc. de biol.*, 1934 117 248.
60. Holman, E., The significance of temporary elevation of blood pressure following splenectomy with particular reference to the role of the spleen as a regulator of the circulation. *Surgery* 1937 1 688.
61. Knœfel, P. K., Anesthesia and the sympathetic nervous system. *Anesth. and Analg.*, 1936 15 137
62. Labat, G. Regional Anesthesia. *Nelson's Loose Leaf Surgery* Thomas Nelson and Sons New York, 1931
63. Levy S. E., and Blalock, A., The effects of unilateral nephrectomy on the renal blood flow and oxygen consumption of unanesthetized dogs. *Am. J. Physiol.*, 1938 122, 609
64. Ludwig W., Untersuchungen zur Frage der Blutdruckregulation. *Arch. f. exper. Path. u. Pharmacol.* 1932, 160 302.
65. Lundy J. S., Adequate and properly controlled artificial respiration for surgical patients by means of a new pulmonary ventilator. *Proc. Staff Meet., Mayo Clin.* 1932 7 225
- 65a. McDonough F. K., Homeostasis in the sympathetomized dog. *Am. J. Physiol.*, 1939 125 530
66. McDowall R. J. S., The nervous control of the blood vessels. *Physiol. Rev.*, 1935 15 98.
67. Maxson, Louis H., Spinal Anesthesia. *J. B. Lippincott Co.*, New York, 1938.
- 67a. Meyer F., and Spiegelhoff W. Der Einfluss peripher angreifender Kreislaufmittel auf Herzleistung, arteriellen Windkessel und Stromungswiderstand. *Arch. f. exper. Path. u. Pharmacol.*, 1938, 190, 256
68. Mitchell, G. A. G., The innervation of the kidney ureter testicle, and epididymis. *J. Anat.*, 1935 70, 10.
69. Morton J. J., and Scott, W. J. M. Studies on the activity of the lumbar sympathetic nervous system. *Ann. Surg.*, 1930 92, 919
70. Nowak, S. J. G., The urinary excretion of novocain after spinal anesthesia and the theory of toxic absorption. *Anesth. and Analg.*, 1933 12, 232.

- 71 Nowak, S J G, and Samaan, A, The effect of adrenaline, anaemia and carbon dioxide on the vasomotor centre. *Arch internat. de pharmacodyn. et de therap.* 1935, 51, 463
- 72 Opitz, E, and Smith, D H, Nierendurchblutung bei Reizung des Carotis-Sinus. *Arch f d. ges Physiol*, 1937, 238, 633
- 73 Pinkston, J O, Partington, P F, and Rosenblueth, A., A further study of reflex changes of blood pressure in completely sympathectomized animals. *Am. J Physiol.*, 1936, 115, 711
- 74 Polano, H, Experimentelle Untersuchungen über das Verhalten des Minutenvolumens des menschlichen Herzens bei Athernarkose, Lumbalanästhesie und nach operativen Eingriffen. *Deutsche Ztschr f Chir*, 1933, 239, 505
- 75 Raab, W Die Beziehungen zwischen CO₂-Spannung und Blutdruck bei Normalen und Hypertonikern. Beitrag zur Pathogenese der nicht "nephritischen" Hypertonien. *Ztschr f d. ges exper Med.*, 1929, 68, 337
- 76 Rakeiten, N, Himwich, H E, and DuBois, D, Morphine acidosis. *J Pharmacol and Exper Therap*, 1934, 52, 437
- 77 Rein, H, and Rossler, R, Die Abhängigkeit der vasomotorischen Blutdruckregulation bei akuten Blutverlusten von den thermoregulatorischen Blutverschiebungen im Gesamtkreislaufe. *Ztschr f Biol*, 1929, 89, 237
- 78 Rhoads, C P, Van Slyke, D D, Hiller, A, and Alvung, A S, The effects of novocainization and total section of the nerves of the renal pedicle on renal blood flow and function. *Am. J Physiol*, 1934, 110, 392.
- 79 Rosenblueth, A, and Cannon, W B., A further study of vasodilators in sympathectomized animals. *Am. J Physiol*, 1934, 108, 599
- 80 Rowntree, L G., and Adson, A W, Further studies on the effects of sympathetic ganglionectomy and ramisectomy. *J A M A.*, 1929, 93, 179
- 81 Schilf, E., and Ziegner, H, Das Wesen der Blutdrucksenkung bei der Lumbalanästhesie. *Arch f klin. Chir.*, 1924, 130, 352.
- 82 Schlossberg, T, and Sawyer, M E MacK., Studies of homeostasis in normal, sympathectomized and ergotaminized animals. IV The effect of hemorrhage. *Am. J Physiol*, 1933, 104, 195
- 83 Schneider, M, and Wildbolz, E., Dekapsulation und Enervation der Niere und Nierendurchblutung. *Ztschr f urol. Chir*, 1937, 43, 1
- 84 Schretzenmayr, A., Über kreislaufregulatorische Vorgänge bei der Nierentätigkeit. *Ztschr f d. ges exper Med.*, 1933, 92, 367
- 85 Schroeder, H A., and Cohn, A. E., Reaction of renal blood flow to partial constriction of the renal artery. *J Clin. Invest. (Proc.)*, 1938 17, 515
- 86 Schubert, O O, On the distribution of the circulation in spinal anesthesia. *Acta. chir Scandinav.*, 1936 78, Supplement 48 1
- 87 Sebrechts, J., Spinal anesthesia with regulation of dosage and author's technique. *Brit J Anaesth.*, 1934, 12, 4
- 88 SeEVERS, M H, and Waters R M, Circulatory changes during spinal anesthesia. *California and West. Med*, 1931, 35, 169
- 89 SeEVERS, M H, and Waters, R M, Respiratory and circulatory changes during spinal anesthesia. *J A M A*, 1932, 99, 961
- 90 Shaw, J L, Steele, B F, and Lamb C A, Effect of anesthesia on the blood oxygen. I A study of the effect of ether anesthesia on the oxygen in the arterial and in the venous blood. *Arch Surg*, 1937, 35, 1
- 91 Shaw, J L, Steele, B F, and Lamb, C. A, Effect of anesthesia on the blood oxygen. II A study of the effect of spinal anesthesia on the oxygen in the arterial and in the venous blood. *Arch. Surg*, 1937, 35, 503
- 92 Smith, H W, The Physiology of the kidney. Oxford University Press, New York, 1937
- 93 Smith, H W, Goldring, W, and Chasis, H, The measurement of the tubular excretory mass, effective blood flow and filtration rate in the normal human kidney. *J Clin Invest*, 1938, 17, 263
- 94 Smith, G G, and Porter, W T, Spinal anesthesia in the cat. *Am J Physiol*, 1915, 38, 108
- 95 Springorum, P W, Über die Unabhängigkeit hormonaler und zentralnervöser Diuresehemmung von der Nierengesamtdurchblutung und dem arteriellen Druck. *Arch. f d. ges Physiol*, 1938, 240, 342.
- 96 Springorum, P W, and Centenera, D, Die verschiedene Beteiligung beider Nieren an Diureseänderungen und vasomotorischen Reaktionen. *Arch. f d. ges Physiol*, 1937, 239, 440
- 97 Starr, I, Gamble, C. J, Margolies, A, Donal, J S, Jr, Joseph, N, and Eagle, E, A clinical study of the action of 10 commonly used drugs on cardiac output, work and size, on respiration, on metabolic rate and on the electrocardiogram. *J Clin Invest*, 1937, 16, 799
- 98 Steel, W A, Blood pressure maintenance in spinal anesthesia. *J A M A*, 1925, 84, 79
- 99 Stierlen, G, Untersuchungen über die Nierengefäßreaktion bei Mängeldurchblutung. *Arch f d. ges Physiol*, 1937, 238, 727
- 100 Theobald, G W, and Verney, E B, The inhibition of water diuresis by afferent nerve stimuli after complete denervation of the kidney. *J Physiol*, 1935, 83, 341
- 101 Thomas, C. B, and Broofs, C. M, The effect of sympathectomy on the vasomotor carotid sinus reflexes of the cat. *Am J Physiol*, 1937, 120, 195
- 102 Unna, K., Arterieller Druck und Nierendurchblutung. *Arch f d. ges Physiol*, 1935, 235, 515
- 103 van Esveld, L. W., Pharmakologie des Vasomotorenzentrums I Der Einfluss einiger Narkotika

- auf die Erregbarkeit des Vasomotorenzentrums für CO_2 . Arch. f. exper. Path. u. Pharmacol., 1930 147 297
- 104 van Esveld, L. W. Pharmacologie des Vasomotorenzentrums II. Der Anteil des Herzens und Vasomotorenzentrums an durch niedrige CO_2 Konzentrationen hervorgerufenen Blutdrucksteigerungen. Arch. f. exper. Path. u. Pharmacol., 1930 147 317
- 105 Verney E. B., and Vogt M. An experimental investigation into hypertension of renal origin with some observations on convulsive "uraemia." Quart. J. Exper. Physiol., 1938 28, 253
- 105a Wezler K., and Böger A., Der arterielle Gesamtwiderstand unter verschiedenartigen Sympathicusreizen. Arch. f. exper. Path. u. Pharmacol., 1937 187 65
106. White, J. C., The Autonomic Nervous System. The Macmillan Company New York, 1935.
- 107 White, J. C., Progress in the surgery of the autonomic nervous system. New England J. Med., 1937 217 660

THE ELIMINATION OF CHOLIC ACIDS IV IN PATIENTS WITH LIVER DISEASES¹

By BERTIL JOSEPHSON

(From the Department of Physiological Chemistry of Karolinska Institutet and from the Medical Department II of Serafimerlasarettet Stockholm Sweden)

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This investigation was undertaken to study the rate of elimination of injected bile salts from the blood of patients with different types of liver disease.

In normal human subjects Josephson and Larsson (13) found that intravenously injected cholate disappeared from the blood very rapidly. Five minutes after the injection only a small part of the injected amount could be found in the blood and after thirty minutes the cholate concentration in the blood was normal again. In one hour most of the injected cholate had been excreted by the liver.

Corresponding results with normal animals had been previously reported by several investigators. This literature was briefly reviewed by Josephson, Jungner, and Rydin (11). On the other hand Snell, Greene, and Rowntree (22) found that in animals with experimental obstructive jaundice disappearance of injected bile salts from the blood was very much delayed. Similar results were obtained later by Bollman and Mann (3) and by Chabrol Cottet and Sallet (6). Jungner, Rydin and Josephson (14) studied the elimination from the blood of sodium cholate injected intravenously into animals with different experimental liver injuries. They found that the rate of disappearance was different in various kinds of jaundice. The elevation of the blood concentration after a cholate injection was as one would expect, much greater in experimental jaundice than in normals. In most cases however the subsequent decrease was rather rapid in obstructive jaundice, but delayed in toxic hepatitis. The present investigation was carried out in order to determine whether corresponding results could be found in patients with liver diseases.

The spontaneous cholic acid concentration in the blood of patients with liver diseases has been the subject of many investigations (2 5 15 16

17 21). All these workers used different methods and their results do not correspond. Usually cases of obstructive jaundice seem to have shown a high cholate concentration while cases with hepatitis and cirrhosis have given widely varying results. For this reason, determinations of the bile salt concentration in the blood have never been of clinical use.

Nakagawa, Simuro and Suzuki (19) have tried to get a bile acid tolerance test by giving patients an injection of sodium dehydrocholate with subsequent analyses of the urine on substances precipitable by acetic acid. Their method, however is not sufficiently specific to yield accurate data concerning bile acid excretion.

METHODS

The experiments were carried out on 62 patients in both medical departments of the Serafimer Hospital. They were divided into two groups. The first includes all cases with evidence of liver or bile duct involvement. The second group consists of control cases who had diseases in which liver involvement usually does not occur.

In the elimination tests pure sodium cholate was used. Ten ml. of a 0.52 per cent solution corresponding to 0.5 gram cholic acid was injected into a cubital vein. The solution also contained 25 per cent glucose which prevents the pain usually caused by an intravenous injection of a pure cholate solution. No pains or symptoms were ever observed in connection with the injections. One blood sample for cholate analysis was taken before the injection. The subsequent samples were taken 5 30 and 60 minutes after the injection in a vein on the opposite side than that where the injection was made. The cholic acid analyses were carried out with the method of Josephson (10)* as modified by Josephson and Larsson (13).

* This method has been criticized by Jenke (9) who has claimed that it would be nonspecific at low concentrations.

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TABLE II
Cholic acids elimination in patients with tumors in the liver or bile ducts

Case number	Patient number	Sex	Diagnosis	Age, years	Diagnosis verified	Skin jaundice	Liver enlargement	Serum bilirubin, mg. per 100 ml.	Icteric index	Galbl. excretion	Tubulin test	Urine			Post-hepatic jaundice	Cholic acids in blood						Differences											
												Bile pig. reaction	Hays test	Urobilin		Before injection	Minutes after injection			I	II		III	IV	V	VI							
																	5	30	60														
1	375/37	M	Bile duct carcinoma liver metastases, acute cholecystitis with multiple liver abscesses	66	p.m.	+	++ nodular	4.8	50*	0.9		+++	+	+++	0	4.9	6.2	4.4	5.5	1.8													
2	762/37	F	Operated breast carcinoma liver and lung metastases	59		++	+++ nodular		65			+	+	0	0	5.4	9.2	7.0	6.4	2.2													
3	1020/37	F	Carcinoma of the gallbladder and bile ducts liver metastases Sept 3	57	p.m.	+++	+++	20.5	60	1.0		++	+	+++	0	6.4	10.1	7.6	7.1	2.5													
4	1089/37	M	Colon carcinoma enormous liver metastases	51	p.m.	+++	+++	100	0.2			++	+	+++	0	8.4	12.2	9.3	9.1	2.9													
5	1212/37	F	Carcinomatosis of liver	43	p.m.	0	+	1.3	15	1.3		0	0	0	+	2.4	4.1	2.7	2.2	1.4													
6	1290/37	M	Liver carcinoma lung metastases, ascites	54	p.m.	++	+	12.1	50			+++				2.7	8.5	3.2	4.9	5.3													
7	524/38	M	Pancreas carcinoma	50	X rays	+++	+++ lobed	0.9	5		Neg	0	0		+	2.0	5.9	3.0	0.8	2.9													
8	600/38	F	Bile duct carcinoma purulent cholangitis	41	p.m.	+++	+++	13.7	45*	0.5		+	+	+	0	7.7	18.0	14.0	8.3	4.0													
9	1176/38	M	Syphilitic tertiary bile duct carcinoma	67		++	smooth	13.6	60*	1.4	Neg	++	++	+	0	1.9	2.6	0.3		2.3													

* Value increasing
 † p.m. = postmortem

The tests were carried out between 9 and 12 a.m. and all subjects were in the postabsorptive state. At the same time as the first blood sample for cholate analysis was taken other samples were drawn for the analysis of bilirubin (Jendrassik and Csike) and of the icterus index (Meulengracht). In addition to the routine tests on bile pigments and urobilin in the urine and feces and the Hay test in the urine, the galactose test (Brauer) was carried out in most cases and frequently also the Takata test. Other liver and blood tests of interest are not reported in the tables as they were carried out only occasionally.

RESULTS

A brief summary of the results obtained from the 27 control cases is given in Table I, showing that there was no marked difference between the

TABLE I

Patients without liver diseases. Concentration of cholic acids in blood before and after injection of 0.5 gram of cholic acid

	Cholate concentration in blood				Difference between concentrations *		
	I Before injec- tion	Minutes after injection					
		5	30	60			
		II	III	IV	II-I	III-III	III-I
	mgm per 100 ml	mgm per 100 ml	mgm per 100 ml	mgm per 100 ml			
Average	2 23	4 40	2 47	2 47	2 06	1 93	0 21
σ	0 84				1 23	1 19	0 65
Maximum	4 0	6 7	4 7	4 6	4 8	4 2	+1 8
Minimum	0 9	2 2	0 9	0 2	0 4	0 2	-1 0

* The sums of and differences between the different averages do not correspond correctly as one value is missing in a few series.

and erroneous at high. According to Josephson's studies (10), it is very probable that the method is specific for cholic acids in blood, but it may be admitted that this is not definitely proved. In this investigation and those of Josephson and collaborators, a nonspecific reaction has no influence on the results, as they deal with comparisons of the concentration over a short period of time in single individuals after addition of pure cholates. For higher concentrations of a degree occurring in jaundice before and after cholate injections, the method has been shown by Josephson to give very satisfactory results. This has been confirmed in this laboratory.

elimination rate in these cases and that in normal cases receiving twice this amount of cholate, as described by Josephson and Larsson (13). The elevation of the blood cholates 5 minutes after the injection was much less than would be expected by dilution in the total volume of the blood. The rate of disappearance of the cholates was more rapid than that of other liver active substances, e.g., bromsulphalein (18). As the 5-minute samples were taken during the rapid decrease of the blood cholates it was quite natural that the concentrations found, and the differences between these and other values should show great variations. After 30 minutes the cholate concentration was normal again.

Three of these cases showing values within the normal limits were treated for severe thyrotoxicosis. This may be of interest in connection with an investigation by Schmidt (20) showing that in rabbits treated with thyroxin, the elimination of injected cholates was considerably delayed. It is also well known that other liver function tests often give pathological values in cases of hyperthyroidism (literature reviewed by Bartels (1)).

The results from the patients with liver symptoms are shown in Tables II to V.

To some degree the original cholate concentration in the blood of these patients corresponded to the degree of jaundice, but there were several exceptions. No difference could be found between the concentration in different kinds of jaundice. In the cases with carcinoma, cirrhosis, or congestion of the liver without jaundice there was no elevation. The concentration was also normal in a few cases with jaundice. The tests in Cases 14, 15, and 20 with hepatitis were carried out during the recovery periods which explains the normal values.

The elevation of the cholates after the injection varied widely but was increased in jaundice and highest in the most jaundiced cases.

The subsequent decrease showed interesting characteristics. In cases with liver tumors the decrease was nearly as rapid as in normals. As a rule, the difference between the 5-minute and the 30-minute values was above 2.2 mgm per 100 ml. There were two exceptions. Case 1, in addition to the obstructive jaundice, had an acute cholangitis with multiple abscesses and extensive destruction of the liver parenchyma, and Case 4 had no

signs of biliary obstruction and a normal elimination curve.

In acute hepatitis the decrease was much slower in Case 11 the difference between the 5-minute and the 30-minute value was 1.1 and in Case 12 1.4 but in other cases it was not over 0.9 mgm per 100 ml. Case 20 with subchronic hepatitis later developed a biliary cirrhosis with ascites.

Two cases of cholecystitis and the cases with liver edema showed normal elimination curves. One case of cirrhosis with severe jaundice had an elimination curve of the hepatitis type. Other cases with cirrhosis reacted normally.

The elimination curves did not correspond to the other liver tests.

The sharp difference between the elimination curves in jaundice due to obstruction and in hepatitis corresponds very well to the results of Jungner, Rydin and Josephson (14). This difference was found even more regularly in human cases of liver diseases than it was in the animals with experimental jaundice. It is possible that the first disappearance from the blood of the major part of the injected cholates was due to adsorption of the bile acids to tissues other than the liver. The subsequent sharp decrease in cases of obstruction could be referred to absorption by a still functioning liver parenchyma, according to Bollman and Mann (4) and Chabrol, Cottet, and Sallet (7). Following this first decrease a slight increase one hour after the injection was observed in some cases. This could be due to a partly maintained circulation of the bile salts by the aid of the lymph vessels of the liver as in experimental obstructive jaundice (12). The existence of such a short circuit of the bile circulation in obstructive jaundice is also supported by the recent experiments of Doubllet (8) who recovered bile pigments in the thoracic lymph of dogs a few minutes after applying slight pressure in the bile ducts. In hepatitis the absorbing function of the liver is diminished and consequently those bile salts which are not adsorbed by other tissues remain in the blood and the decrease is slow.

SUMMARY

In jaundice, the increase of the cholic acid content in the blood after an injection of sodium cholate is greater than in normals. It is not

greater in liver diseases without jaundice. The subsequent decrease is delayed in acute hepatitis but not in jaundice due to obstruction of the bile ducts. A cholic acid elimination test seems to be valuable in the differential diagnosis between these two kinds of jaundice.

BIBLIOGRAPHY

1. Bartels, E., Liver function in hyperthyroidism as determined by the bippuric acid test. *Ann. Int. Med.*, 1938, 12, 652.
2. Boku, S. and Gon K., Studien über die Gallensäuren über das Verhalten der Blutgallensäuren bei Leber und Gallengangskrankheiten. *J. Chosen M. A.* 1933, 23, 87.
3. Bollman, J., and Mann, F., Alterations in hepatic function produced by experimental hepatic lesions. *Ann. Int. Med.* 1935, 9, 617.
4. Bollman, J., and Mann, F., The influence of the liver on the formation and destruction of bile salts. *Am. J. Physiol.*, 1936, 116, 214.
5. Chabrol, E., Charonnat, R., and Cottet, J., La recherche des sels biliaires dans le sérum sanguin par la réaction phospho-vanilique. *Compt. rend. Soc. de biol.*, 1934, 115, 835.
6. Chabrol, E., Cottet, J., and Sallet, J., Recherches comparatives sur le pouvoir de concentration du foie et du rein vis à vis de l'acide cholalique. *Compt. rend. Soc. de biol.*, 1936, 122, 184.
7. Chabrol, E., Cottet, J., and Sallet, J., Recherches sur l'enrichissement du foie et du muscle en acide cholalique au cours des cholalétries expérimentales. *Compt. rend. Soc. de biol.* 1936, 122, 186.
8. Doubllet, H., Personal communication, 1939.
9. Jenke, M., Zwei neue spektrochemische Verfahren zum Nachweis und zur quantitativen Bestimmung von Gallensäuren in Blut, Stuhl und Urin. *Verhandl. d. deutsch. Gesellsch. f. inn. Med.*, 1937, 49, 246.
10. Josephson, B., The determination of cholic acids in blood. *Biochem. J.*, 1935, 29, 1519.
11. Josephson, B., Jungner, G., and Rydin, A., Elimination of cholic acids. I. In healthy animals. *Acta med. Scandinav.*, 1938, 97, 237.
12. Josephson, B., and Kaunitz, H., Die Resorption der Gallensäuren bei experimentellem Icterus. *Ztschr. f. d. ges. exper. Med.*, 1937, 102, 195.
13. Josephson, B. and Larsson, H., Elimination of cholic acids. III. In man. *Acta med. Scandinav.*, 1939, 99, 140.
14. Jungner, G., Rydin, A., and Josephson, B., Elimination of cholic acids. II. In experimental jaundice. *Acta med. Scandinav.*, 1938, 97, 254.
15. Katayama, I., Bile acids in jaundice. *Arch. Int. Med.* 1928, 42, 916.
16. Kaunitz, H., and Kent, H., Ueber die klinische Bedeutung von Veränderungen der Oberflächenspannung.

TABLE V
Elimination of cholic acids in patients with secondary liver symptoms

Case number	Patient number	Sex	Diagnosis	Age	Diagnosis verified	Skin jaundice	Liver enlargement	Serum bilirubin	Icterus index	Gallstone excretion	Takata test	Urine			Feces bile pigments	Cholic acids in blood				Difference
												Bile pigments	Days test	Urobilin		Before injection	Minutes after injection			
																	5	30	60	
				years				mgm per 100 ml		grams					mgm per 100 ml	mgm per 100 ml	mgm per 100 ml	mgm per 100 ml	mgm per 100 ml	
29	497/37	M	Myeloid leukemia, enormous liver enlargement	77		0	++	0.8	4			0	0	0	+	17	24	0.9	1.1	1.5
30	1055/37	M	Heart failure, liver enlargement	56		0	++		4			0	0	0	++	27	4.9	2.7		2.2
31	172/39	M	Heart failure, liver enlargement	63		0	++			0.4		0	0	0	+	16	5.1		2.7	3.0
32	142/39	M	Heart failure, liver enlargement	42		0	+					0	0	0	+	4.9	7.7	1.7		
33	635/38	F	Heart failure, liver enlargement	46	p m	+	++	2.4	16	0.3		0	0	0	+	3.5	6.5	3.8	1.7	2.7
34	653/38	M	Pyelonephritis, uremia, liver enlargement	49		0	++	0.1	1			0	0	0	+	3.8	5.6	4.2	2.7	1.4
35	766/38	M	Pancreatic carcinoma, ascites	67	X ray	0	0	1.8	9			0	0	0	±	4.3	5.2	4.6	3.6	0.6

† p m = postmortem

THE ORIGIN AND NATURE OF NORMAL SYNOVIAL FLUID¹

By MARIAN W. ROPES, GRANVILLE A. BENNETT, AND WALTER BAUER

(From the Medical Clinic of the Massachusetts General Hospital, the Departments of Medicine and Pathology, Harvard Medical School, and the Massachusetts Department of Public Health, Boston)

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The physical and chemical properties of normal synovial fluid have never been well established. In consequence there exists no uniformity of opinion concerning its mode of formation. If we possessed information concerning the origin and nature of normal synovial fluid we would be in a position to interpret more correctly the abnormalities encountered in pathological joint effusions and to determine their diagnostic significance. Information of this type should also increase our knowledge of the factors involved in the production and maintenance of joint effusions.

In 1691, Havers (62), on the basis of histological examinations, concluded that synovial fluid was a secretion from synovial membrane glands. Since then various descriptions of synovial fluid and theories concerning its origin have appeared. This lack of agreement is readily explained if one examines the data upon which the various theories are based. Some of them are based solely on histological studies. Others represent conclusions drawn from chemical analyses of pathological synovial fluids. The data on pathological fluids, many of which are incomplete, vary markedly and are difficult to interpret without knowledge of the normal and a better understanding of the factors responsible for the formation of pathological fluids. The existing data pertaining to normal synovial fluid are very meagre, except for complete cytological studies (6, 73, 122).

The various theories proposed and the data on which they are based are presented in brief.

1 That synovial fluid is the secretory product of synovial membrane cells or glands. This theory originally proposed by Havers (62) and supported by many subsequent workers (4, 11,

16, 25, 52, 65, 70, 77, 85, 93, 99, 103, 105, 107, 112) is based chiefly on histological examinations of synovial membrane. Drawings or photomicrographs of such glands have never been presented.

2 That synovial fluid is chiefly the product of secretion by synovial membrane cells with the addition of a transudate from the capillaries and lymphatics (78, 90). This theory is for the most part based on histological studies. More recently Kling (77), on the basis of certain physical and chemical measurements of normal and pathological synovial fluids, concluded (1) that normal synovial fluid is secreted by the synovial membrane (2) that pathological synovial fluid contains both secretory and circulatory products.

3 That synovial fluid is a mixture of the products of disintegration of synovial membrane rubbed off during joint motion and a transudate from the capillaries and lymphatics. This theory presented by Frerichs in 1846 (45) has been supported in a modified form by other workers (1, 21, 31, 35, 56, 57, 59, 100, 110). Here again histological studies serve as the chief basis for such conclusions.

4 That synovial fluid is formed as a result of destruction of cartilage because of constant use. Originally proposed by Ogston (97) and Bianchi (5), this theory has received no support except for the statement by Fisher (35) in which he suggested that a portion of the synovial fluid mucin might be derived from articular cartilage as it becomes worn.

5 That synovial fluid is a dialysate from the blood capillaries. This theory was first suggested by Bichat (12) in 1812. He concluded that the glands described by Havers were fat deposits and that synovial fluid is formed directly by exhalation of the blood capillaries. This theory has been proposed by many workers (2, 8, 13, 20, 27, 46, 64, 69, 72). More recent reviews (98, 102) of the existing data on synovial fluid have led to the conclusion that synovial fluid is in ready

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- nung des Blutserums. *Ztschr f klin. Med.*, 1936, 131, 235
- 17 Lichtmar S., A new procedure for the estimation of bile salts in body fluids based on bile salt hemolysis. *J Biol. Chem.*, 1934, 107, 717
 - 18 Mills, M., and Dragstedt C., Removal of intravenously injected bromsulphalein from the blood stream of the dog. *Arch. Int. Med.*, 1938, 62, 216
 - 19 Nakagawa, S., Simuro, S., and Suzuki, S., Gallensäurebelastungsprobe zur Leberfunktionsprüfung. *Klin. Wchnschr.*, 1934, 13, 1392.
 - 20 Schmidt L. H., The removal of sodium cholate from the blood and its secretion into the bile as affected by thyroxine. *Am J Physiol.*, 1937, 120, 75
 - 21 Snell, A. Clinical aspects of portal cirrhosis. *Ann. Int. Med.*, 1931, 5, 333
 - 22 Snell, A., Greene, C., and Rowntree, L., Diseases of the liver—further studies in experimental obstructive jaundice. *Arch. Int. Med.*, 1927, 40, 471
 - 23 Snell, A., and Magath, T., The use and interpretation of tests for liver function. *J. A. M. A.*, 1933, 110, 167

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In 1691 Havers (62) on the basis of histological examinations, concluded that synovial fluid was a secretion from synovial membrane glands. Since then various descriptions of synovial fluid and theories concerning its origin have appeared. This lack of agreement is readily explained if one examines the data upon which the various theories are based. Some of them are based solely on histological studies. Others represent conclusions drawn from chemical analyses of pathological synovial fluids. The data on pathological fluids, many of which are incomplete, vary markedly and are difficult to interpret without knowledge of the normal and a better understanding of the factors responsible for the formation of pathological fluids. The existing data pertaining to normal synovial fluid are very meagre, except for complete cytological studies (6, 73, 122).

The various theories proposed and the data on which they are based are presented in brief.

1 That synovial fluid is the secretory product of synovial membrane cells or glands. This theory originally proposed by Havers (62) and supported by many subsequent workers (4, 11,

16, 25, 52, 65, 70, 77, 85, 93, 99, 103, 105, 107, 112) is based chiefly on histological examinations of synovial membrane. Drawings or photomicrographs of such glands have never been presented.

2 That synovial fluid is chiefly the product of secretion by synovial membrane cells with the addition of a transudate from the capillaries and lymphatics (78, 90). This theory is for the most part based on histological studies. More recently Kling (77), on the basis of certain physical and chemical measurements of normal and pathological synovial fluids, concluded (1) that normal synovial fluid is secreted by the synovial membrane, (2) that pathological synovial fluid contains both secretory and circulatory products.

3 That synovial fluid is a mixture of the products of disintegration of synovial membrane rubbed off during joint motion and a transudate from the capillaries and lymphatics. This theory, presented by French in 1846 (45), has been supported in a modified form by other workers (1, 21, 31, 35, 56, 57, 59, 100, 110). Here again histological studies serve as the chief basis for such conclusions.

4 That synovial fluid is formed as a result of destruction of cartilage because of constant use. Originally proposed by Ogston (97) and Banchi (5) this theory has received no support except for the statement by Fisher (35), in which he suggested that a portion of the synovial fluid mucin might be derived from articular cartilage as it becomes worn.

5 That synovial fluid is a dialysate from the blood capillaries. This theory was first suggested by Bichat (12) in 1812. He concluded that the 'glands' described by Havers were fat deposits and that synovial fluid is formed directly by 'exhalation' of the blood capillaries. This theory has been proposed by many workers (2, 8, 13, 20, 27, 46, 64, 69, 72). More recent reviews (98, 102) of the existing data on synovial fluid have led to the conclusion that synovial fluid is in ready

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diffusion equilibrium with plasma and except for the presence of mucin would be considered a diffusate or a simple ultrafiltrate of serum. Such conclusions are based on histological studies or on incomplete chemical analyses of pathological synovial fluids except for those of Peters (98) which represent conclusions based on our data.

6 That synovial fluid is the specialized fluid matrix of a specialized connective tissue lining an enlarged tissue space, the joint cavity (23, 68, 71, 75). According to this theory, which is based on analogy with no experimental evidence except histological studies, the synovial fluid mucin corresponds to the mucoid constituent of other connective tissues. The conception of mucin as the ground substance of synovial tissue (116) is in accord with this theory.

Discussion of these theories at this time is unnecessary. It is sufficient to state that no one of them has gained general acceptance because knowledge of the physical and chemical properties of synovial fluid has been insufficient to allow one to speak with certainty concerning its origin or its nature.

In the present investigation, extensive physical and chemical analyses of simultaneously obtained arterial blood and normal synovial fluid were made. It was hoped that a complete characterization of normal synovial fluid, a comparison of the distribution ratios of electrolytes and non-electrolytes between serum and fluid with those for other body fluids and *in vivo* dialysates and anatomical studies would allow us to conclude which one of the previously mentioned theories is correct.

In smaller laboratory animals and in man, normal synovial fluid is present in such small amounts that aspiration is difficult. Furthermore, analyses of such small quantities of synovial fluid would necessitate the use of microchemical methods. These difficulties are readily overcome if one resorts to young western cattle because the astragalotibial joint contains a large amount of readily available normal synovial fluid. Therefore, the present studies were made on arterial blood and synovial fluid obtained from young western cattle immediately after they had been slaughtered. It was impossible to have the animals under standard conditions. During the week previous to slaughter, they had been transported in cattle cars and presumably had stood for abnormally long periods

of time. At the time of slaughter, they were fasting and had not been at rest.

The synovial fluid was aspirated from the astragalotibial joint under paraffin oil. The amount of fluid obtained from a single joint varied between 20 and 50 cc. Any obviously abnormal fluids (presumably produced by previous trauma) such as blood-tinged, deep yellow, or turbid fluids were discarded. Blood was obtained from the carotid arteries under paraffin oil. No anticoagulant was employed. The blood and the fluid were kept on ice until centrifuged, some 60 or 80 minutes later.

The total quantity of synovial fluid obtained from these animals may have been greater than normal because the animals had stood for abnormally long periods of time in transit East. In man under such conditions, transudation of an essentially protein-free fluid from the blood capillaries into the tissues of the leg takes place (80, 84, 111, 125). If similar transudation of an essentially protein-free filtrate occurred in these cattle, some dilution of the normal synovial fluid may have resulted. Membrane equilibrium, if present, should be maintained, however, despite the increased amount of fluid.

The following chemical methods were used: chloride, Eisenmann modification of the Van Slyke method (30), carbon dioxide content, Van Slyke and Neill (114), inorganic phosphate, Fiske and Subbarow (40), sodium, Rourke's modification of the Kramer-Gittleman method (101), potassium, Fiske and Litarczek (37), calcium, Fiske and Logan (38), magnesium, Fiske and Logan (39), nonprotein nitrogen, Folin and Wu (42), uric acid, Benedict and Behre (9), urea, Lieboff and Kalin (86), sugar, Folin (41), total base, Fiske (36), sulphate, Fiske (36), freezing point, Beckmann (34), fatty acids, Stoddard and Drury (108), cholesterol, Bloor (14), lactic acid, Friedemann, Cotonio, and Shaffer (47), osmotic pressure was determined by the Krogh method (79, 81), using collodion membranes which were made according to Krogh's

* We are indebted to Dr. D. F. Loeven for the osmotic pressure readings, to Dr. C. Daley for the determination of the temperature coefficients, to Miss D. Sloane for the freezing point determinations, and to Mrs. D. Gilligan and Miss M. Rourke for the potassium estimations.

directions and had minute numbers from 100 to 200 as described by Zsigmondy (126). The pH was determined by means of a McInnis glass electrode, measurements being made at 25° to 28° and corrected to 37° by the use of a temperature coefficient. The temperature coefficient for the pH of synovial fluid was found to be approximately half that for serum ($\Delta\text{pH}/\Delta t = 0.006$ for synovial fluid, 0.012 for serum). In the first fifteen cases, the pH was not determined but was calculated from the Henderson Hasselbalch equation. The carbon dioxide tension used in the equation was estimated from the carbon dioxide content and the carbon dioxide absorption curves (109). Specific gravity was determined by the use of specific gravity bottles with open capillary outlet of the type described by Moore and Van Slyke (94). Total solids were determined by drying a weighed sample of approximately 1 gram at 100° C. for 48 hours. Viscosity was determined both by a Hess viscosimeter and by an Ostwald viscosity pipette.

The protein other than mucin was calculated from the total nitrogen (obtained by a modified macro-Kjeldahl method). The difference between the total nitrogen and the sum of the non protein and mucin nitrogen was multiplied by the factor 6.25. Mucin was determined by precipitation with 1 per cent acetic acid and reprecipitation with acetic acid from a 0.1 per cent sodium carbonate solution. The difference in total nitrogen before and after precipitation, representing the mucin nitrogen was converted to mucin by the factor, 8.14 (the factor of 8.14 has been obtained in this laboratory by analysis of pure mucin). In cases in which the mucin was not determined, an estimated mucin nitrogen of 0.015 grams per 100 cc. was used for calculation of the protein. Albumin and globulin contents were determined both by a modification of the Howe method (67) and by the method of Butler and Montgomery (18).

RESULTS

Fifteen joint fluids and sera were analyzed in great detail and 45 other fluids and sera were analyzed in part. In Table I are given the values for cytology and chemical composition in the fifteen fluids analyzed in detail. The chemical constituents are expressed in concentration for each

1000 grams of water in the serum or fluid. In these fifteen cases the water content was obtained from determinations of the total solids and specific gravity. Calculated values for the water content of these same fluids were obtained for comparison by the use of the formula $W = 99.6 - 0.85 P$ where W is the water content in grams per 100 cc. and P represents the grams of protein per 100 cc. The calculated values also are given in Table I and are seen to be in close agreement with the observed values. In fluids in which the water content was not determined directly it was calculated from the above formula. In calculating the equivalent bicarbonate, $\frac{1}{2} \text{H}_2\text{O}$ of the carbon dioxide content of the fluid or serum was assumed to represent free carbonic acid. The proportions of primary acid phosphate, $\text{B}_2\text{H}_2\text{PO}_4$, and secondary phosphate B_2HPO_4 , in the serum and the fluid were calculated from the Henderson formula

$$\text{pH} = \text{pK}' + \log \frac{\text{B}_2\text{HPO}_4}{\text{B}_2\text{H}_2\text{PO}_4},$$

using the value for pK' in blood as 6.8

The results will be presented under the headings of cytology, physical characteristics protein constituents, distribution of non-electrolytes enzymes, and distribution of electrolytes. In each case the findings will be compared with those found by other workers for synovial fluid and other body fluids. The results will be analyzed with the aim of determining whether they provide evidence that would indicate whether or not synovial fluid is a dialysate in equilibrium with blood plasma. Experiments with dialysis *in vitro* are, in general, not acceptable as evidence in the solution of this problem because all such experiments are open to question since absolute physiological conditions are not reproduced. Therefore, the problem can be studied best by a determination of the distribution of substances between arterial serum and synovial fluid and a comparison with the ratios expected in accord with the known physicochemical laws of equilibrium across semi permeable membranes. Further evidence can be obtained by a comparison of the distribution ratios with those of the same substances between serum and the *in vivo* dialysate (55) and between serum and other body fluids which have been shown to have the composition of dialysates of blood plasma (lymph and edema fluids). Recent studies on

the nature of lymph and edema fluids have given results in accord with those obtained by dialysis and with those expected from the laws of membrane equilibrium. Recent workers agree that the concentrations of inorganic constituents of lymph and edema fluids are such that these fluids may be regarded as ultrafiltrates or dialysates in equilibrium with plasma. (See review of subject by Landis (83).)

Cytology

Normal cattle synovial fluid is relatively acellular. The average nucleated cell count of this series is 131 cells per cu mm, with a maximum of 250 and a minimum of 65. The erythrocyte counts show an average of 194, with a maximum of 1540 and a minimum of 0. These values are in accord with those obtained in two large series of fluids from normal cattle joints (6, 122)—the average nucleated cell counts in these series being 112 and 182 respectively and the average red blood cell counts being 64 and 141. Differential leukocyte counts were not done in this present study as the series of Warren, Bennett, and Bauer (122) had established the average differential counts in fluid from the astragalotibial joints of normal cattle and had indicated the wide variations in phagocytic and non-phagocytic cell percentages which may occur in normal joints. They reported an average differential nucleated cell count of polymorphonuclear leukocytes, 22 per cent, monocytes, 36.4 per cent, clasmatoocytes, 15 per cent, unclassified phagocytes, 3.9 per cent, lymphocytes, 40.1 per cent, synovial cells, 1.2 per cent, unclassified cells, 1.2 per cent.

Few other workers have studied the cytology of normal fluid. Key (73) studied fluid from the shoulder joints of rabbits and found 175 to 225 nucleated cells per cu mm and approximately the same number of red blood cells (74). The differential nucleated cell count was: synovial lining cells, 3 per cent, primitive cells (resembling small lymphocytes), 1 per cent, polymorphonuclear leukocytes, 5 per cent, monocytes, 58 per cent, clasmatoocytes, 15 per cent and indeterminate phagocytes, 14 per cent. Labor and Von Balogh (82) found 10 to 20 cells per cu. mm in fluid obtained postmortem from human joints, and McEwen (91), in two normal human fluids, found total nucleated cell counts of 125 and 200 per cu

mm. In the fluid from knee joints of amputated legs, Kling (76) found 10 to 50 cells per cu mm. Forkner (43) from a review of previous studies assumed that normal synovial fluid contained ± 50 nucleated cells per cu mm.

The cytology of 29 fluids obtained postmortem from human joints showing no evidence of disease has been studied in this laboratory (127). The average nucleated cell count was 63 per cu mm with an average differential count: polymorphonuclear leukocytes, 6.5 per cent, monocytes, 47.6 per cent, clasmatoocytes, 10.1 per cent, unclassified phagocytes, 4.9 per cent, lymphocytes, 24.6 per cent, synovial cells, 4.3 per cent, unclassified cells, 2.2 per cent.

Physical characteristics

Normal synovial fluid is a clear, straw-colored, viscous liquid, which does not clot.

The average specific gravity is 1.010 with a maximum of 1.012 and a minimum of 1.009. These figures correspond with those found by Horiye (1.008 to 1.015) (66) for human fluids obtained postmortem from joints in which no histological changes were found in the membrane. They are in the same range also with the values found by Gilligan, Volk, and Blumgart (49) for chest fluid (1.010 to 1.015) and edema fluid (1.008 to 1.009). The total protein calculated from the average specific gravity of this series by use of the formula of Moore and Van Slyke (94) is 1.029 grams per 100 cc, which is in close agreement with the observed average protein content (including mucin) of 1.02 grams per 100 cc.

The average content of total solids is 2.084 grams per 100 grams with a maximum of 3.886 and a minimum of 1.672, as compared with an average content of 8.727 in the serum. The fluid values correspond closely with those of Horiye (66) for human fluids obtained postmortem (1.20 to 3.93 per cent). They are slightly lower than the value given by Fisher (35) for normal human fluids obtained postmortem (4.4 per cent).

The average freezing point of the fluid is -0.535° with a maximum of -0.556° and a minimum of -0.509° , as compared with an average for the serum of -0.590° (as shown below). The average difference between the freezing point depression of the fluid and that of the serum (0.0550) is much greater than that found

TABLE 1†
Cytology and chemical composition of normal cattle serum and synovial fluid

Cow number	Cytology		Water (Observed)		Water (Calculated)		Protein (Not including mucin)		pH†		Chloride		Bicarbonate		Phosphorus		Total bases		Calcium		Sugar		Nonprotein nitrogen		Urea	
	W.B.C.	R.B.C.	cells per cu. mm.	grams per 100 c.c.	FI	Se	FI	Se	FI	Se	FI	Se	FI	Se	FI	Se	FI	Se	FI	Se	FI	Se	FI	Se	FI	Se
I	70	63	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	20	20	12.5	12.5
II	85	30	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	19	19	8.9	8.9
III	75	75	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	21	21	7.4	7.4
IV	85	15	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	21	21	7.4	7.4
V	85	15	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	21	21	7.4	7.4
VI	150	150	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	21	21	7.4	7.4
VII	150	200	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	21	21	7.4	7.4
VIII	130	200	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	21	21	7.4	7.4
IX	170	150	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	17	17	8.9	8.9
X	170	150	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	17	17	8.9	8.9
XI	150	275	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	17	17	8.9	8.9
XII	150	350	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	17	17	8.9	8.9
XIII	125	675	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	18	18	7.1	7.1
XIV	125	675	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	18	18	7.1	7.1
XV	210	240	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	21	21	7.4	7.4
Average	131	164	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	21	21	8.5	8.5
Maximal	250	1310	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	31	31	12.8	12.8
Minimal	65	0	91.04	92.07	7.31	7.35	106.3	106.3	23.3	23.3	1.4	1.4	15.62	15.62	8.8	8.8	3.7	3.7	25	25	12.5	12.5	15	15	6.8	6.8
Number of fluids*	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31

* This represents the number of fluids from which the averages were obtained

† Figures in this table were calculated from chart based on the Henderson Hasselbalch equation

‡ In this and subsequent tables, Se = serum FI = synovial fluid

by Fremont-Smith, Thomas, Dailey, and Carroll (44) between normal spinal fluid and blood. In 62 per cent of their cases the difference was 0.005° or less, and in only 21 per cent was it greater than 0.015°, the maximum difference being only 0.037°. The observed freezing points of serum and synovial fluid represent a difference in osmolar concentration of 0.030 μ , and a difference in pressure of approximately 0.7 atmospheres. It is unlikely that such a difference in osmolar concentration exists. A probable explanation is that the determination of the freezing point of synovial fluid is affected in some unknown manner by the presence of the viscous mucin.

Cow number	Freezing point	
	Se °C	Fl °C
XVI	-0.586	-0.524
XVII	-0.587	-0.547
XVIII	-0.581	-0.540
XIX	-0.616	-0.556
XX	-0.549	-0.509
XXI	-0.622	-0.536
Average	-0.590	-0.535
Maximal	-0.616	-0.556
Minimal	-0.549	-0.509

The average relative viscosity at 25° C is 3.72, with variations from 2.84 to 4.15. The viscosity is due chiefly to the presence of mucin, as shown by the fact that a value of 1.1 is obtained following precipitation of the mucin. Studies of the viscosity of normal synovial fluids have been reported only in the case of humans. Determinations made in this laboratory indicate that the viscosity is much higher in normal human fluid. Schneider (104) reported variations from 3.9 to 14.90 in fluids obtained postmortem from patients who had had no joint disease.

The average osmotic pressure against Ringer-Locke solution is 365 mm of water for the serum and 150 mm of water for the fluid (see Table II). The average osmotic pressure difference between the fluid and the serum determined directly is 250 mm of water. It is of significance to compare these values with the colloidal osmotic pressure values calculated from the average albumin and globulin figures for our series, using the factors determined by Govaerts (53) for the pressure exerted per gram by serum albumin (75.4) and serum globulin (19.5). The osmotic pres-

sure of the serum, calculated in this way, is 384 mm of water which agrees fairly well with the observed value of 365 mm. In the case of the fluid, however, the colloidal osmotic pressure calculated from the albumin and globulin content is only 57 mm of water, in contrast to the observed value of 150 mm. The other known colloidal substance in the fluid is the mucin. Little is known of the osmotic pressure exerted by mucin. If one assumes that the difference between the observed and the calculated osmotic pressures of the fluid is due to mucin, and calculates the osmotic pressure effect per gram of mucin, the value is nine times as great as that for albumin (675 as compared with 75).

TABLE II
Colloidal osmotic pressure of normal cattle serum and synovial fluid

Cow number	Protein (Not including mucin)		Osmotic pressure in Ringers		Osmotic pressure serum vs fluid
	Se	Fl	Se	Fl	
	grams per 100 grams H ₂ O	grams per 100 grams H ₂ O	mm H ₂ O	mm H ₂ O	mm H ₂ O
XXII	7.97	1.249	354	170	261
XXIII	8.16	0.771	384	142	257
XXIV	7.92	0.854	365	147	261
XXV	7.95	0.688	352	141	223
XXVI	7.57	0.688	347	127	241
XXVII	7.89	0.803	379	145	
XXVIII	8.48	1.288	403	163	
XXIX	7.62	0.656	340	139	
XXX	7.63	0.904	352	167	
XXXI	8.08	1.103	378	160	
Average			365	150	249
Maximal			403	170	261
Minimal			340	127	223

Protein constituents

The average concentration of protein in the fluid, including the mucoprotein, is 1.02 grams per 100 grams of water, in contrast to 7.87 grams per 100 grams of water in the serum. This figure is in the same range as the few values that have been reported for normal synovial fluid and somewhat lower than the values found for lymph and edema fluids. Fisher (35) found the protein of normal human fluid 1.6 per cent and of fluid from oxen 0.92 per cent. Horie (66) found variations from 0.45 to 3.15 per cent in the protein content of fluids obtained postmortem from joints in which he found no histological changes in the membrane. From a relatively large experience

with fluids obtained postmortem from humans without joint disease and fluids from pathological human joints, we would conclude that the value of 3.15 per cent reported by Horie represents an abnormal fluid. Cajori and Pemberton (20), in one synovial fluid from a patient with generalized edema, found a protein of 1.39 per cent. Heim (63) reported variations from 1.38 to 4.57 per cent in the total protein of lymph, while Arnold and Mendel (3) found 3.56 per cent protein in lymph. The total protein concentrations of the fluids studied by Gilligan, Volk, and Blumgart (49) varied from 0.25 gram per 100 grams of water in one subcutaneous edema fluid to 4.36 grams in a case of ascites secondary to carcinoma. Their average value for all fluid proteins (chest, ascitic, and edema fluids) was 1.49 grams per 100 grams of water.

The content of albumin and globulin in our series is of the same general magnitude as that found by other workers for normal synovial fluid and somewhat lower than that of other fluids which have been shown to be dialysates of blood plasma. The presence of serum proteins in lymph and other body fluids has never been explained adequately. The majority of investigators (22, 24, 28, 32, 80, 121) have concluded that the proteins result from a slight generalized capillary permeability to protein and subsequent concentra-

tion. Other workers (83, 84, 89, 111) do not agree with these findings and have concluded that capillary permeability to proteins is negligible. The possibility of formation of the proteins *in situ* may be another factor but has never been investigated. The summation of evidence at present indicates that there is a slight permeability of normal capillary walls to proteins. There is no general agreement as to whether or not the permeability is sufficient to explain the concentration of protein in body fluids.

Approximately one-eighth of the fluid protein (0.138 gram per 100 grams of water) is mucin. It is this mucoprotein that produces most of the viscosity and the resulting lubricating value of the fluid and, as has been discussed above, it is presumably this mucoprotein that causes part of the excessively high osmotic pressure of the fluid. By precipitation with acetic acid and reprecipitation from sodium carbonate solution we have obtained a pure mucin the composition and characteristics of which we shall report in a subsequent publication. The concentration of mucin found by us can be compared with the figures given by French (45) for the mucin of cattle fluid. He found 0.326 per cent in fluid from newborn calves, 0.24 per cent in fluid from oxen kept in stalls for long periods and 0.56 per cent in fluid from oxen allowed free in pastures. Von Holst (117)

TABLE III
Concentrations of the protein constituents of normal cattle serum and synovial fluid

Cow number	Protein (Not including mucin)		A/G ratio† by Na ₂ SO ₄		A/G ratio† by PO ₄		Albumin† by Na ₂ SO ₄		Albumin† by PO ₄		Mucin
	Se	Fl	Se	Fl	Se	Fl	Se	Fl	Se	Fl	
	grams per 100 grams H ₂ O	grams per 100 grams H ₂ O					grams per 100 grams H ₂ O	grams per 100 grams H ₂ O	grams per 100 grams H ₂ O	grams per 100 grams H ₂ O	
XXXII	7.75	0.594	1.28	3.81	0.98	4.89	4.36	0.470	3.83	0.493	0.138
XXXIII	8.20	0.743	1.25	1.51	1.00		4.55	0.448	4.10		
XXXIV	7.33	0.713	1.17	1.57	0.98	2.48	3.96	0.436	3.64	0.508	0.098
XXXV	7.85	1.203	1.18	2.50	1.02	2.82	4.24	0.860	3.95	0.889	0.212
XXXVI	7.33	1.017	1.28	2.51	1.22	3.97	4.11	0.727	4.03	0.812	0.147
XXXVII	7.47	0.911	1.38	2.59	1.21	5.82	4.32	0.657	4.09	0.777	0.252
XXXVIII	7.38	1.023	1.61	2.89	1.27	3.40	4.55	0.761	4.14	0.791	0.155
Average	7.87	0.886	1.31	2.48	1.10	3.90	4.30	0.623	3.97	0.712	0.138
Maximal	8.75	1.410	1.61	3.81	1.27	5.82	4.55	0.860	4.14	0.889	0.252
Minimal	7.11	0.435	1.17	1.51	0.98	2.48	3.96	0.436	3.64	0.493	0.033
Number of fluids *	37	36	7	7	7	6	7	7	7	6	11

* This represents the number of fluids from which the averages were obtained

† Method of Howe (67)

‡ Method of Butler and Montgomery (18)

found 0.5 per cent mucin in normal cattle fluid. Fisher (35) found 1.95 per cent mucin in normal human fluid and only 0.13 per cent in fluid from oxen. Cajori and Pemberton (20) report a mucin content of 0.42 per cent in fluid from a patient with generalized edema.

The globulin fraction of the fluid protein, as determined by precipitation with 22.5 per cent sodium sulphate, averages 0.26 gram per 100 grams of water, with an average albumin content of 0.62 gram per 100 grams of water. (See Table III.) The average albumin-globulin ratio of the fluid is 2.5, in contrast to an average ratio of 1.3 in the serum. When the protein fractions are determined by the method of Butler and Montgomery (18), using a 2.3 M solution of phosphate, the value obtained for the albumin fraction of serum is lower than that obtained by sodium sulphate precipitation. The resulting albumin-globulin ratio is somewhat lower (1.1). This is in accord with the results of the two methods as reported by Butler and Montgomery. In the case of the fluid, however, the albumin concentration determined by precipitation with phosphate is consistently higher than that obtained by precipitation with sodium sulphate. This finding is presumably due to the loss of albumin during the precipitation of globulin by sulphate, which has been shown to occur when the total protein content is low (50, 67). Experiments performed in this laboratory with dilutions of serum have shown that no such loss of albumin occurs with phosphate precipitation. The albumin-globulin ratio of the fluid as determined by precipitation with phosphate is, therefore, higher (3.9) than that obtained by precipitation with sulphate (2.5), and gives a more accurate representation of the protein fractions as accepted at present.

In all of the determinations of the protein fractions, the mucin nitrogen was subtracted from the difference between the total protein nitrogen and albumin nitrogen in order to obtain the globulin nitrogen. The accuracy of the albumin concentration obtained by precipitation with either sulphate or phosphate solutions even in a mucin-containing solution has been proved in this laboratory by precipitation experiments on solutions of pure mucin in serum. These experiments have shown that mucin is precipitated with the globulin

fraction of the fluid when either sulphate or phosphate solutions are used.

The globulin concentration and the albumin-globulin ratio varied more in the fluid than in the serum, as was found in the case of pathological fluids by Cajori and Pemberton (20). Similarly, marked variations in the albumin-globulin ratio in other body fluids were found by Gilligan, Volk, and Blumgart (49). This may be due in part to less accuracy in separation of the protein fractions when the total protein content is low, and in part to slight variations in capillary permeability.

The comparatively low globulin concentration and high albumin-globulin ratio of the fluid in contrast to those of the serum is in accord with the results found by Field, Leigh, Heim, and Drinker (29, 33) for lymph and edema fluid, and with the findings of Wells (124), and Weech, Goettsch, and Reeves (123) and of Goettsch and Kendall (50) in lymph, edema fluid, and ascitic fluid. Assuming that the serum proteins of the fluids result from slight capillary permeability, the high albumin-globulin ratio in the fluids indicates greater capillary permeability to albumin than to globulin. The difference is in accord with the difference in molecular weights of albumin and globulin, and with the variation in their rates of removal from joints (7).

Normal fluid contains no fibrinogen as suggested by the failure to clot after standing twenty-four hours. The absence of fibrinogen has been corroborated in this laboratory by precipitation experiments with 1.1 M phosphate solutions at pH 6.5.

Distribution of non-electrolytes

The average concentration of urea in the fluid (expressed in milligrams per 100 grams of water) is slightly lower than that in the serum but is essentially of the magnitude that would be expected if serum and fluid were separated by a membrane permeable to this substance. The distribution ratios of total nonprotein nitrogen (0.87) and uric acid (0.84) between fluid and serum are somewhat lower than that of urea, but they are of the same general magnitude. These findings are in accord with those reported by Hare and Cohen (58) for normal horse syno-

vial fluid, and by other workers for pathological fluids (2 19 20, 23 96)

Although the average distribution ratios for urea, uric acid, and nonprotein nitrogen between fluid and serum are slightly below 1 00 analysis of the results of determinations in individual sera and fluids shows many cases in which the concentrations of these non-electrolytes are as high in the fluid as in the serum (see Table I and figures given below) These individual cases in themselves give proof that the non-electrolytes (urea, uric acid and nonprotein nitrogen) are completely diffusible through the membrane separating serum and fluid We have found further evidence of the complete diffusibility of these non-electrolytes in our results on normal and pathological human fluids which will be reported in a later publication. In the majority of those cases, the distribution ratio of nonprotein nitrogen and uric acid between fluid and serum closely approaches 1 00

Cow number	Se	Uric acid	Fl
	mgm. per 100 grams H ₂ O		mgm. per 100 grams H ₂ O
XXXXII	2 10		2 08
XXXXIII	2 26		1 95
XXXXIV	2 04		1 60
XXXXV	1 67		1 20
XXXXVI	1 54		1 37
XXXXVII	1 70		1 30
XXXXVIII	1 59		1 35
Average	1 84		1 55
Maximal	2 26		2 08
Minimal	1 54		1 20

The average concentration of sugar in the present series is on the other hand, much lower in the fluid than in the serum. The values in individual cases vary much more than those for any other substance. The marked variations in values and the lower concentration in the fluid may be due in part to the fact that the animals were not fasting, and in part to the fact that they struggled considerably when sacrificed thereby raising the concentration of sugar in the serum just before the samples were collected and not allowing time for the fluid to come to equilibrium with the serum. That this is the explanation rather than the presence of a non-diffusible portion of glucose in the serum as suggested by Brull (17) is apparent from our findings in human fluids which

will be reported in detail in a later publication. In many of these cases, the distribution ratios of sugar between fluid and serum closely approach 1 00. This is in accord with the results of Walker and Reisinger (120) who found complete diffusion of reducing substances between plasma and glomerular urine.

Cholesterol and fatty acids are absent in the fluid. This is in accord with the generally accepted theory that the capillary membrane under normal conditions is not permeable to these substances.

Thus the distribution of non-electrolytes is consistent with the theory that synovial fluid is a dialysate of blood plasma.

Enzymes

Except for the determination of the phosphatase activity of fluid and serum, no enzyme studies were undertaken. The fluids were found to have a higher average phosphatase activity than the serum. Greater variations were encountered in the fluids. Further studies are needed in order to explain such variations.

Distribution of electrolytes

The concentrations of chloride and bicarbonate are higher in the fluid than in the serum while the concentrations of sodium, potassium, calcium, and magnesium are lower in the fluid than in the serum. The concentration of total inorganic phosphate is practically the same in fluid and serum. These distributions, are, in general such as would be expected from consideration of the laws regulating membrane equilibrium. They will be analyzed in detail below.

The excess of chloride in the fluid bears about the same relation to the excess of protein in the serum as has been found by other workers for lymph, edema fluids and the *in vivo* dialysate. The relationship may be graphically presented as was done by Gilligan, Volk, and Blumgart (48). The excess of chloride in the fluid in proportion to the excess of protein in the serum, however is slightly lower than that found for the other fluids (Chart I). This may be related to the nature and relative concentration of the proteins in synovial fluid. The high albumin globulin ratio in the fluid would tend to increase the base-binding power per gram of total.

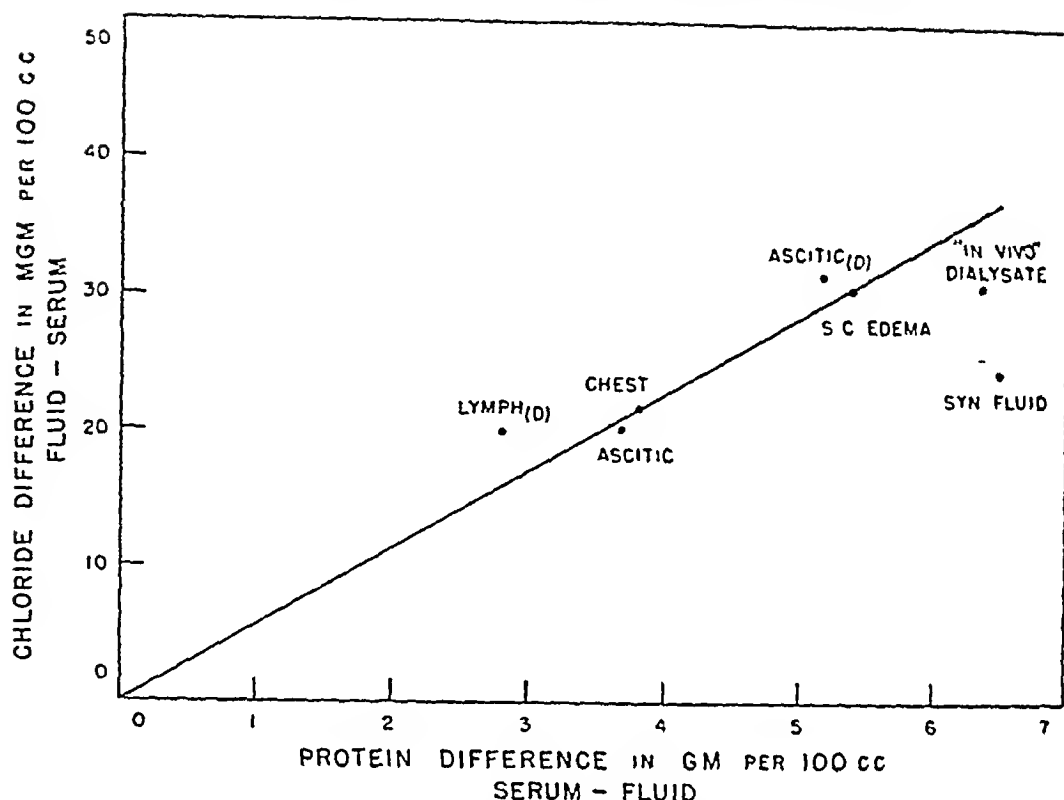


CHART I THE RELATIONSHIP BETWEEN THE DIFFERENCE IN CHLORIDE CONCENTRATION OF VARIOUS BODY FLUIDS AND SERUM AND THE DIFFERENCE IN THEIR PROTEIN CONCENTRATIONS

The values charted represent average values from the following studies: chest fluid, total of 14 observations (49, 54, 87), lymph, dogs, total of 7 observations (3, 63), ascitic fluid, total of 18 observations (49, 54, 61, 87), ascitic fluid, dogs, total of 10 observations (54), subcutaneous edema fluids, total of 16 observations (49, 51, 61), *in vivo* dialysates, dogs, total of 15 observations (55), synovial fluid, total of 15 observations from our studies.

presence of mucin may further increase the base-binding power, as indicated by the results of solubility experiments on pure mucin now in progress. In addition, the isoelectric point of mucin (approximately pH 4.0) is farther from the pH of fluid than are the isoelectric points of albumin or globulin with the result that at pH 7.4 it has a considerable degree of combination with base.

Hydrogen-ion concentration

The average pH of the fluid is 7.31 as compared with an average pH of 7.42 for the serum. The averages include the values calculated from the Henderson-Hasselbalch equation (Table I) and the values determined by means of the glass electrode (listed below). Few reports of the pH of normal synovial fluid have been made. Horiye (66) found fluid obtained postmortem from human joints to be weakly alkaline to litmus.

Seeliger (106) reported the pH of postmortem fluid as 8.2 to 8.4. Boots and Cullen (15) found a pH of 7.34 in fluid from a patient with generalized edema.

Cow number	Se	pH	Fl
XXXII	7.55		7.43
XXXIII	7.49		7.39
XXXIV	7.47		7.39
XXXV	7.52		7.31
XXXVI	7.45		7.27
XXXVII	7.42		7.27
XXXVIII	7.40		7.35

The pH of 7.31 found in the synovial fluid is of interest. In other body fluids which have the composition of dialysates the fluid pH has been found to be slightly higher (0.02 to 0.05 unit) than that of the serum (61, 87). In the case of synovial fluid the pH is 0.11 unit lower than the

serum pH The low pH of the fluid is associated with an exceptionally high carbon dioxide tension (an average of 58.8 mm., as shown in the tabulation below) The corresponding pH and carbon dioxide tension values of the serum were in the normal range The explanation of the values found in the fluid is not apparent

Cow number	Carbon dioxide tension Se	mm
I	48.8	59.1
II	48.9	56.8
III	46.4	58.9
IV	51.1	50.4
V	49.5	
VI	37.4	55.3
VII	36.1	53.6
VIII		62.5
IX		55.6
X		59.7
XI		58.0
XII	41.4	51.7
XIII	44.1	70.1
XIV	44.3	67.4
XV	40.2	64.6
Average	44.4	58.8
Maximal	51.1	70.1
Minimal	36.1	50.4

Application of the Gibbs Donnan theory of membrane equilibrium

The significance of this theory as applied to the equilibrium between plasma and body fluids is recognized even though the inability to determine with any degree of accuracy either the activities of ions in so complex a system or the presence of other modifying factors makes it impossible to obtain exact mathematical agreement between calculated and observed values By this theory the distribution of ions is expressed mathematically by the equation

$$r = \frac{(Cl)_s}{(Cl)_f} = \frac{(HCO_3)_s}{(HCO_3)_f} = \frac{(Na)_s}{(Na)_f} = \frac{(K)_s}{(K)_f} \text{ etc}$$

These equations hold strictly only when expressed in terms of activities of the various ions However they can be assumed to be valid when expressed in terms of concentrations if we assume that the diffusible salts are ionized to an equal extent in serum and fluid and that the activity coefficients of the ions in the two fluids are not significantly different

In order to determine whether our results are in accord with the Donnan theory the approxi-

mate Donnan distribution ratio has been calculated using the formula derived by Van Slyke Wu, and McLean (115) and estimating the base bound by protein by means of the formula of Van Slyke, Hastings Hiller and Sendroy (113) The theoretical average Donnan ratio for our studies is 0.933 The distribution ratios determined by us are compared in Table IV with this theoretical Donnan ratio and with the ratios found by Greene and Power (55) for *in vivo* dialyses and those found by the following workers for various body fluids Gilligan Volk and Blumgart (49) Gollwitzer Meier (51) and Loeb Atchley and Palmer (87) edema fluid Arnold and Mendel (3) and Heim (63) lymph Darrow Hopper and Carey (26) Greene Bollman Keith and Wakefield (54) Muntwyler, Way and Pomerehne (95) ascitic fluid Hastings Salvesen Sendroy and Van Slyke (61) ascitic and edema fluids

The average distribution ratios between serum and synovial fluid of chloride, bicarbonate inorganic phosphate sulphate and total anions as found in our studies are all of the same order of magnitude

The average ratio $\frac{(Cl)_s}{(Cl)_f}$ is 0.99 which conforms fairly well with those found for other fluids It is 6 per cent higher than the average Donnan ratio a deviation from the theoretical approximately the same as that found by Greene and Power (55) in the case of the *in vivo* dialysate. The discrepancy may be, as they suggest due in part to the fact that the base proteinate is not completely ionized as is assumed in calculating the theoretical Donnan ratio It may however be due in part also to the high albumin globulin ratio and the mucin content of the fluid both of which tend to increase the base binding power per gram of total protein.

The average ratio $\frac{(HCO_3)_s}{(HCO_3)_f}$ is 0.94 which is in close agreement with the theoretical Donnan ratio and conforms fairly well with the bicarbonate ratio found for other fluids Deviation from the chloride ratio may depend on several factors The bicarbonate ratio represents that between arterial blood and fluid and as would be expected this ratio has been found by various workers to be lower than that between

TABLE IV
Comparison of distribution ratios between serum and tissue body fluids

Ratio	Preparation	Conc. of Cl ⁻ (40) Plasma fluids	Conc. of P ₂ O ₅ (50) Plasma fluids	Conc. of Cl ⁻ (50) Venous fluid	Conc. of Cl ⁻ (50) Transudates	Conc. of Water-Miscible (50) Plasma fluids	Van Slyke from Lock (50) Plasma fluids	Hem. (50) Lymph	Arterial and Venous (50) Lymph	Haworth et al. (1)		M. W. F. et al. (2)	
		Ca ²⁺	Human	Dog	Human	Human	Human	Dog	Dog	Human	Human	Human	Human
Cl _i /Cl _f	0.65	0.65	0.65	0.97	0.97	0.91	0.67	0.95	0.95	0.97	1.01	0.97	0.97
HCO _{3i} /HCO _{3f}	0.91	1.01 (ven) 0.91 (art)	0.97	0.93	1.03	0.95	1.05 (ven) 0.95 (art)			0.97	0.97	1.05	1.05 (ven) 1.05 (art)
PO _{4i} /PO _{4f}	1.09	1.03	1.17	1.12	1.05			0.95	1.19				
Na _f /Na _i	0.93	0.95	0.91	0.91	0.95		1.01			0.91	0.93		
K _f /K _i	0.75	0.81	0.78	0.91	0.75	0.75	0.60			0.71			
Ca _f /Ca _i	0.65	0.70	0.58	0.71	0.79			0.81					
$\sqrt{\frac{Ca_f}{Ca_i}}$	0.83		0.76	0.83	0.87					0.80	0.85		
Mg _f /Mg _i	0.72		0.61	0.77	0.99								
$\sqrt{\frac{Mg_f}{Mg_i}}$	0.85		0.66	0.86	0.99								
Theoretical Donnan	0.933	0.955	0.933				0.97			0.962	0.975	0.957	0.959 (ven) 0.951 (art)

blood and fluid (see Table IV). Furthermore, the discrepancy may be due in part to variation in the carbon dioxide content of blood from the carotid artery and blood from capillaries around the knee. In addition, true equilibrium probably never exists because carbon dioxide is constantly being poured into the fluids from the tissues to be removed by the blood (98).

The average ratio $\frac{(\text{lactic acid})_s}{(\text{lactic acid})_f}$ is 2.11. The concentrations in individual cases show marked variations (as shown below). The extremely high distribution ratio and the variations in con-

centrations in individual sera and fluids are presumably explicable as in the case of the sugar by the fact that the animals struggled considerably when sacrificed, thereby raising the lactic acid concentration in the blood and not allowing time for the fluid to come to equilibrium with the blood.

The average ratio $\sqrt{\frac{(SO_4)_s}{(SO_4)_f}}$ is 1.06, 7 per cent higher than the chloride ratio. Since the determination of sulphate in blood and fluid is not exact, the 7 per cent deviation is not of great significance, and the sulphate ratio may be con-

Cow number	Lactic acid	
	Se	fl
	m.eq. per 1000 grams H ₂ O	m.eq. per 1000 grams H ₂ O
XVI		3.80
XVII	8.18	3.45
XVIII	4.57	3.56
XIX	9.71	3.57
XX	4.14	2.06
XXI	5.93	2.84
Average	5.47	3.21
Maximal	9.71	3.80
Minimal	4.14	2.06

Cow number	Sulphate	
	Se	fl
	m.eq. per 1000 grams H ₂ O	m.eq. per 1000 grams H ₂ O
XVI	6.01	4.75
XVII	5.33	5.01
XVIII	5.31	4.91
XIX	5.40	4.53
XX	5.28	5.11
XXI	6.03	5.42
Average	5.56	4.96
Maximal	6.03	5.42
Minimal	5.28	4.53

sidered in general agreement with the chloride ratio

The average ratio $\frac{(\text{total anions})_s}{(\text{total anions})_f}$ is 0.99, which agrees well with the ratio found for the *in vivo* dialysate (55) and with the chloride ratio in our findings

The average ratio $\frac{(\text{total inorganic phosphate})_s}{(\text{total inorganic phosphate})_f}$ is 1.00. The average ratios of the primary and secondary phosphates are

$$\frac{(\text{H}_2\text{PO}_4)_s}{(\text{H}_2\text{PO}_4)_f} = 0.787 \text{ and } \sqrt{\frac{(\text{HPO}_4)_s}{(\text{HPO}_4)_f}} = 1.03$$

This is in accord with the results of Maly (88) who showed that the acid phosphates are more diffusible. However the most important ratio in a consideration of the diffusibility of phosphate is that of the total inorganic phosphate. There has been considerable variation in the phosphate ratios between serum and dialysates and between serum and body fluids as found by various workers. As a result, there is disagreement as to the proportion of the phosphate of the blood that is diffusible. Greene and Power (55) and Gilligan Volk and Blumgart (49) have concluded that part of the inorganic phosphate is held in the serum presumably bound by protein. Brull (17) in a review of the results obtained by dialysis and vividiffusion experiments points out that the results in general indicate that the phosphate of blood is entirely diffusible. In his own experiments Brull found that the inorganic phosphate of serum is practically entirely diffusible, but that the majority of the inorganic phosphate of heparinized plasma is not ionized and not diffusible. Heim (63), working on lymph, and Walker (118-119) working on lymph and glomerular urine, have concluded that all of the inorganic phosphate of the blood is diffusible. Our results indicate a slightly greater ratio of total inorganic phosphate than the theoretical Donnan ratio but the phosphate ratio is within one per cent of the chloride ratio determined by us and would indicate that the inorganic phosphate is entirely diffusible and that its distribution is determined by the same laws of membrane equilibrium as regulate the distribution of chloride between serum and synovial fluid.

The average distribution ratios between fluid and

serum of sodium potassium calcium and magnesium, in contrast to those for anions vary markedly among themselves but they agree, in general, with the distribution ratios for the same sub-

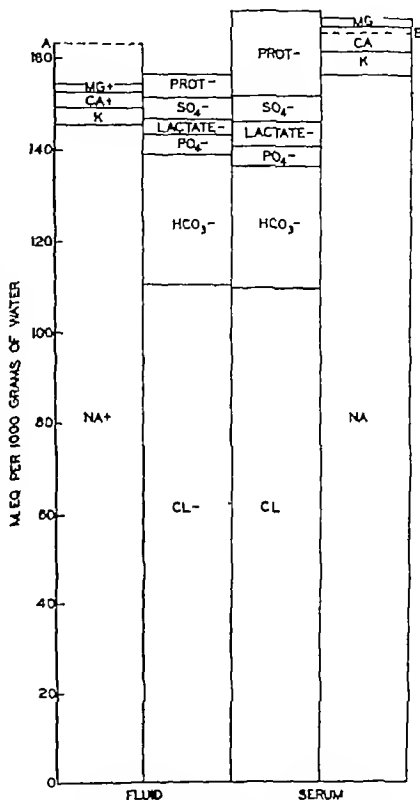


CHART II THE DISTRIBUTION OF ANIONS AND CATIONS BETWEEN SERUM AND NORMAL CATTLE SYNOVIAL FLUID

It will be noted that the summations of individual bases in the fluid and serum do not correspond exactly with the average determined total base values for fluid (A) and serum (B).

The formulae of Van Slyke, Hastings Hiller and Sordroy (113) were used in estimating the proteinate. In the case of mucin the base-binding power was assumed to be ten times the average base-binding power of albumin and globulin.

stances between the *in vivo* dialysate and serum and between lymph and edema fluids and serum.

The average ratio $\frac{(Na) f}{(Na) s}$ is 0.93, which is identical with the theoretical Donnan ratio but slightly lower than the chloride ratio as found in our experiments. It is in fairly good agreement with the ratios found by other workers. The slight deviation from the chloride ratio may indicate that a small percentage (6 per cent) of the sodium is held in the serum in a non-diffusible form, presumably bound to protein. The deviation, however, may not be sufficiently great to be of significance.

The average ratio $\frac{\sqrt{(Ca) f}}{\sqrt{(Ca) s}}$ is 0.83, and indicates as do the similar calcium ratios obtained for the *in vivo* dialysate and other fluids, that part of the calcium is held in the serum presumably bound to protein. This concept of non-diffusible calcium bound to protein is now generally accepted (see review by McLean and Hastings (92)). The percentage of the blood calcium thus bound has been found to be from 30 to 40 per cent. The results of our experiments give an average of 32 per cent bound. Of more significance than the distribution ratio of total calcium is that of ionized calcium. Calculation of the calcium ion in serum and fluid from the protein and total calcium concentrations (McLean and Hastings) gives a ratio $\frac{(Ca^{++}) f}{(Ca^{++}) s}$ of 1.18. This ratio

is much higher than would be expected from the laws of membrane equilibrium. The difference may be explicable in part by the fact that, in calculating the calcium ion concentrations of the serum and fluid, no consideration was given to the difference in pH and albumin-globulin ratios, but in larger part by the fact that the mucoprotein was included as part of the total protein and considered to have the same effect as the serum proteins. That the last assumption is incorrect is evident from a comparison of the calcium concentration of synovial fluid with that of other body fluids known to be dialysates of blood plasma. A review of the results on all such fluids gives an average empirical ratio $\frac{(Ca) \text{ dialysate}}{(Ca^{++}) \text{ serum}}$ of 1.33

(60). Using the average calcium ion concentra-

tion in the serum in our series (1.21 mM per kgm of water) the calcium concentration of synovial fluid calculated from the above empirical formula is 1.61 mM per kgm of water in contrast to the observed value of 1.00 mM. The difference (0.20 mM per kgm of water) represents an estimate of the calcium bound by mucin. In terms of millimols of calcium bound per gram of mucin the figure is 0.23 mM, a value approximately ten times that obtained for serum proteins (92). This is in agreement with the results of our experiments on pure mucin discussed above, which indicate that the base-combining power of mucin is high.

TABLE V
Concentrations of potassium, magnesium and sodium in normal cattle serum and synovial fluid

Cow number	Potassium		Cow number	Magnesium		Cow number	Sodium	
	Se	Fl		Se	Fl		Se	Fl
	m.eq per 1000 grams H ₂ O*	m.eq per 1000 grams H ₂ O		m.eq per 1000 grams H ₂ O*	m.eq per 1000 grams H ₂ O		m.eq per 1000 grams H ₂ O*	m.eq per 1000 grams H ₂ O
XXXI	5.46	4.06	XXIX	1.67	1.37	XXI	115.7	110.1
XXVII	5.12	4.10	XL	1.71	1.33	XXII	132.9	127.3
XXVIII	5.87	4.15	XLI	1.78	1.37	XXIII	156.5	114.2
XXVII	5.57	4.40	XLII	2.22	1.31	XXIV	153.6	111.1
XXVIII	5.45	3.60	XLIII	1.51	1.42	XXV	155.1	115.4
XLIX	4.44	3.00	XLIV	1.78	1.72	XXVI	167.9	116.1
			XLV	1.67	1.45			
			XLVI	1.61	1.35			
Average	5.37	4.01		1.75	1.41		146.1	115.0
Maximal	5.87	4.40		2.22	1.77		167.9	117.3
Minimal	4.44	3.00		1.51	1.33		114.7	109.1

* Calculated with average figures for water content.

The average ratios $\frac{(K) f}{(K) s}$ (0.76) and $\frac{\sqrt{(Mg) f}}{\sqrt{(Mg) s}}$ (0.88) were obtained from a smaller number of analyses, the results of which varied considerably. (See Table V.) However, the deviation from the chloride ratio is great and of the same magnitude as that found by other workers and probably is of significance in spite of the variation in results. One can conclude that part of the potassium (approximately 25 per cent) and part of the magnesium (approximately 30 per cent), as well as part of the calcium, are held in the serum in a non-diffusible state. The variation in results makes it impossible to estimate accurately what proportion is bound in this way.

The average ratio $\frac{(\text{total base}) f}{(\text{total base}) s}$ (0.98) is identical with the chloride ratio. The results of the

individual determinations of the total base concentration however varied markedly. The distribution ratio of total base concentrations obtained by summation of the average concentrations of the individual cations in the fluid and serum is 0.91. This value may be a more accurate indication of the base held in the serum in a non-diffusible state.

Thus the distribution of electrolytes agrees, in general with that expected from the Donnan theory of membrane equilibrium and with the results obtained by Greene and Power (55) in the study of the *in vitro* dialysate and by various workers in the study of other fluids which have been shown to have the composition of dialysates. Therefore the distribution of electrolytes is in accord with the theory that synovial fluids is a dialysate of blood plasma.

Anatomical considerations

Having found that the distribution of electrolytes and non-electrolytes is in accord with the concept that synovial fluid is a dialysate experiments were undertaken to determine whether the vascular supply to the synovial membrane and subsynovial tissues is consistent with this theory.

Employing the same technique previously described (10) the blood vessels of the rear extremities of dogs were perfused with a 6 per cent gum acacia solution. The perfusion was terminated by the injection of a suspension of graptolite. This method made possible the filling of the subsynovial blood vessels with a substance that could be easily recognized on macroscopic and microscopic examination.

Gross examination of a knee joint from a leg so perfused (Figure I) demonstrates the existence of a rich subsynovial blood supply. It will be noted that the most vascular areas are the infrapatellar fat pad and the subsynovial tissue immediately adjacent to the patella. On examination of the microscopic sections taken from this same joint (Figure II) one notes that the subsynovial tissues possess a generous blood supply. One further notes that such blood vessels in many instances are separated from the joint cavity by only a few layers of cells. In other sections it was found that 6 to 20 cells intervene between the joint cavity and the blood vessels. Thus it

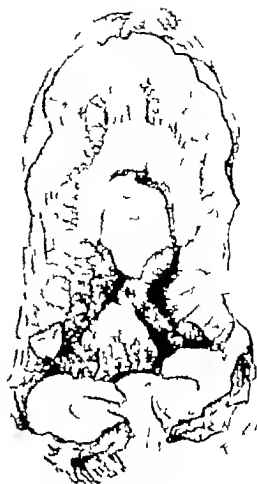


FIG. I. A NATURAL SIZED DRAWING OF THE INTERIOR OF THE LEFT KNEE JOINT OF A NORMAL DOG.

The blood vessels of the rear extremities had been perfused with a suspension of graphite. It will be noted that the subsynovial tissues are very vascular particularly in the region of the infrapatellar fat pad and immediately adjacent to the patella.

would appear that the blood supply to the subsynovial tissues is sufficiently great and so arranged to allow readily for the diffusion of plasma water into the joint cavity. Such anatomical facts lend further support to the interpretation of the chemical findings, namely that synovial fluid is a dialysate.

COMMENT

The distribution of electrolytes and non-electrolytes between serum and normal synovial fluid as well as the nature of the vascular supply of the knee joint, is in accord with the concept that normal synovial fluid is a dialysate in equilibrium with blood plasma. Such a theory explains all known facts of the physical and chemical composition of synovial fluid except the presence of mucin, albumin and globulin. The presence of mucin however in no way invalidates the theory. Whatever the source of the mucin, whether it be the surrounding connective tissue as seems most

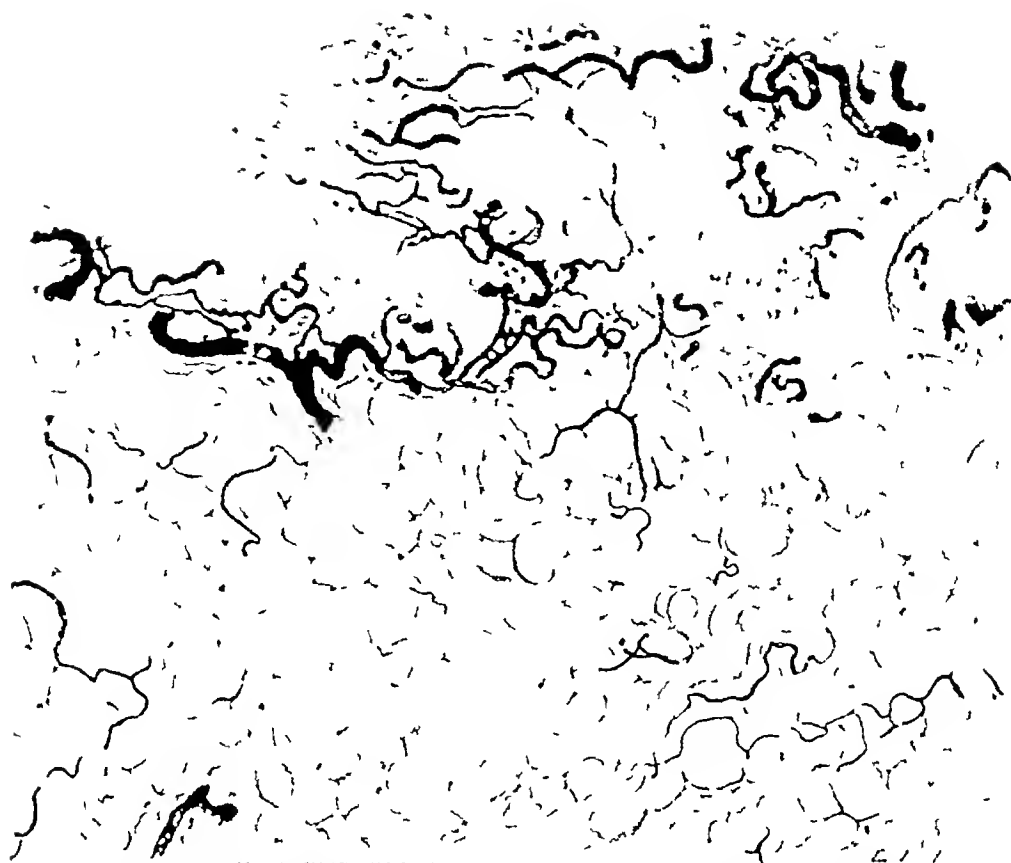


FIG. II. CAMERA LUCIDA DRAWING OF LOW MAGNIFICATION ($\times 100$) OF A MICROSCOPIC SECTION TAKEN FROM THE OPPOSITE KNEE JOINT OF THE ONE SHOWN IN FIGURE I.

The rich subsynovial vascular system is well illustrated. In some instances the blood capillaries are separated from the interior of the joint by not more than one or two layers of synovial lining cells.

likely, or cartilage the synovial fluid itself can be formed by dialysis.

Little is known concerning the source of synovial fluid mucin. Kling (77) considers the phenomenon of sac formation in acetic acid as evidence of the secretory nature of synovial fluid. The absence of sac formation in transudates and exudates and its presence in saliva and synovial fluid merely indicate the absence or presence of a sufficient quantity of a mucin to form a sac, and give no evidence as to whether or not the mucin is of secretory origin. Photomicrographic evidence of synovial membrane glands has never been presented, nor have we ever observed such glands in our studies of synovial membrane. In fact, histological studies show that synovial membrane consists of connective tissue varying in type in different portions of the joint and is not a

specialized membrane. The only evidence favoring the theory that mucin is formed in cartilage is that of a similarity of staining reactions (5). No chemical identity has been established. Other workers have suggested that mucin is secreted by individual cells of the synovial membrane or that it represents the fluid matrix of the specialized connective tissue lining an enlarged tissue space—the joint cavity. Whether the process of mucin formation be described as secretion or matrix formation is immaterial because in either instance connective tissue cell activity is essential. Extraction from the subcutaneous tissue of rabbits and the tissue lining the astragalotibial joints of cattle of a substance similar to synovial fluid mucin as shown by its physical properties and by enzymatic studies (127) suggests that mucin is formed by the connective tissue cells surrounding

the joint. Its entrance into the joint is made possible by the diffusion of plasma water from the underlying vessels through the subsynovial tissue and membrane.

The presence of albumin and globulin in syno-

vial fluid can be explained presumably on the basis of slight capillary permeability to protein as discussed above. Albumin and globulin are found in varying amounts in other body fluids (lymph edema pleural and ascitic fluids) which have been



FIG. III. OTHER PORTIONS OF THE SYNOVIAL LINING TISSUES OF THE JOINT ILLUSTRATED IN FIGURE II ARE SHOWN IN THESE CAMERA LUCIDA DRAWINGS OF HIGH MAGNIFICATION ($\times 420$)

The close proximity of the blood capillaries to the interior of the joint is evident.

shown to have the composition of simple dialysates of blood plasma. The high albumin-globulin ratio in the fluid indicates a greater permeability to albumin than to globulin as suggested also by the observations of other investigators (33, 50, 123, 124).

It is interesting to note that in spite of the fact that synovial fluid unlike other body fluids contains mucin, in most respects it resembles these fluids. The only marked differences between the mucin-containing synovial fluid and the other body fluids that have the composition of dialysates of blood plasma are the high colloid osmotic pressure and the high calcium concentration in synovial fluid. The latter finding can be ascribed, presumably, to the high base-combining power of mucin. These effects of mucin in joint fluid are of significance as an indication that mucin in addition to its action as a lubricant plays a role in the exchange of water and other substances between the vascular system and the joint cavity.

The concept that synovial fluid is a dialysate of blood plasma to which is added mucin as the fluid diffuses through the connective tissue surrounding the joint is not fundamentally different from the concept that synovial fluid represents the fluid matrix of specialized connective tissue, nor does this theory differ materially from that in which synovial fluid is considered a combination of synovial membrane cell secretion with transudation from the capillaries.

The results of the present investigation give experimental evidence for the theory that synovial fluid is a dialysate of blood plasma containing mucin, albumin, and globulin. Additional evidence is found in the results on human fluids obtained postmortem from normal joints. These will be reported later.

SUMMARY

Normal bovine synovial fluid is a relativelyacellular clear, straw-colored, viscous liquid. It has the following characteristics: a nucleated cell count of 131 per c. mm., a relative viscosity of 3.72, a pH of 7.31 as compared with a serum pH of 7.42 (average figures are presented).

The total protein concentration is 1.02 grams per 100 grams of water, of which 0.71 gram per cent is albumin, 0.17 globulin and 0.14 mucin. Fibrinogen is absent.

The distribution of electrolytes and nonelectrolytes between serum and fluid is in accordance with the concept that synovial fluid is a dialysate of blood plasma.

The nature of the subsynovial vascular supply to the knee joint is in accord with this concept.

Unlike other body fluids that are dialysates of blood plasma, synovial fluid contains mucin, the origin of which is unknown. The effect of mucin on the colloid osmotic pressure and calcium concentration of synovial fluid indicates that mucin in addition to its action as a lubricant also plays a role in the exchange of water and other substances between the vascular system and the joint cavity.

BIBLIOGRAPHY

1. Aebys, C. *Ein Lehrbuch der Anatomie. Der Bau des menschlichen Körpers mit besonderer Rücksicht auf seine morphologische und physiologische Bedeutung.* Leipzig, 1868-1871. Cited by Mayeda.
2. Allison, N., Fremont-Smith, F., Druley, M. I., and Kennard, M. A., Comparative studies between synovial fluid and plasma. *J. Bone and Joint Surg.* 1926, 8, 758.
3. Arnold, R. M., and Mendel, L. B. Interrelationship between the chemical composition of the blood and the lymph of the dog. *J. Biol. Chem.*, 1927, 72, 189.
4. Aschoff, L., *Pathologische Anatomie.* II. G. Fischer, Jena, 1911, p. 233.
5. Banchi, A., Ricerche intorno alla struttura della sinoviale, ed alla presunta origine della sinoviale. *Atti d. Accad. med. fis. Firenze.* Firenze 1902, 190, 27.
6. Bauer, W., Bennett, G. A., Marble, A., and Clifton, D. Observations on the normal synovial fluid of cattle. I. The cellular constituents and nitrogen content. *J. Exper. Med.*, 1930, 52, 835.
7. Bauer, W., Short, C. I., and Bennett, G. A. The manner of removal of proteins from normal joint. *J. Exper. Med.*, 1933, 57, 419.
8. Bechard, P. A. *Éléments d'anatomie générale.* Paris 1865, 4th ed. Cited by Hammar and Mayeda.
9. Benedict, S. R., and Behre, J. A. The analysis of whole blood. III. Determination and distribution of uric acid. *J. Biol. Chem.* 1931, 92, 161.
10. Bennett, G. A., Bauer, W., and Maddock, S. J. A study of the repair of articular cartilage and the reaction of normal joints of adult dogs to surgically created defects of articular cartilage, joint mice, and patellar displacement. *Am. J. Path.* 1932, 8, 499.
11. Bernstein, J., *Lehrbuch der Physiologie des tierischen Organismus im Speziellen des Menschen.* Stuttgart 1894. Cited by Mayeda.
12. Bichat, X. *Anatomie Générale Appliquée à la*

- Physiologie et à La Médecine. Paris 1812, 4, part 2 p. 538
13. Beck, E. M., Surgical pathology of synovial tissue. *J Bone and Joint Surg.* 1930 12, 33
14. Bloor W R., The determination of cholesterol in blood. *J Biol. Chem.* 1916 24 227
15. Boots R. H. and Cullen G E., The hydrogen ion concentration of joint exudates in rheumatic fever and other forms of arthritis. *J Exper Med.* 1922, 36, 405
16. Brinton, W in Todd R. B., *Cyclopaedia of Anatomy and Physiology* London, 1847-49 4 part 1 p. 511 Cited by Hammar
17. Brull, L., Contribution à l'Étude de L'état Physico-chimique des Constituants Minéraux et du Glucose Plasmatiques. *Arch. internat. de physiol.* 1930 32, 138.
18. Butler A. M., and Montgomery H., The solubility of the plasma proteins. I Dependence on salt and plasma concentrations in concentrated solutions of potassium phosphate. *J Biol Chem.* 1932, 99, 173.
19. Cajori, F A., Crouter C. Y., and Pemberton, R., The physiology of synovial fluid. *Arch. Int. Med.* 1926, 37 92.
20. Cajori, F A and Pemberton R., The chemical composition of synovial fluid in cases of joint effusion. *J Biol. Chem.* 1928 76 471
21. Cherry J H., and Ghormley R. K., A histopathological study of the synovial membrane with mucicarmine staining *J Bone and Joint Surg.* 1938 20 48.
22. Churchill E. D., Nakazawa, F., and Drinker C. K., The circulation of body fluids in the frog *Am. J Physiol.* 1927 63 304
23. Collins D H., The pathology of synovial effusions *J Path. and Bact.* 1936 42 113
24. Conklin, R., The formation and circulation of lymph in the frog III. The permeability of the capillaries to protein. *Am. J Physiol.* 1930 95 98.
25. Cornil A. V and Ranvier L. *Manuel d'histologie pathologique.* Paris 1901 3d ed., p. 38
26. Darrow D C., Hopper E. B., and Cary M. K., Plasmapheresis edema. II The effect of reduction of serum protein on the electrolyte pattern and calcium concentration. *J Clin. Invest.* 1932, 11, 701
27. Drechsel E., *Chem. der Absonderung und der Gewebe.* Herrmann Handbuch der Physiologie, 1883, 5 Abt. 1 p 617
28. Drinker C. K., and Field, M. E. The protein content of mammalian lymph and the relation of lymph to tissue fluid. *Am. J Physiol.* 1931 97, 32.
29. Drinker C. K., Field, M E., Helm, J W., and Leigh, O C., Jr., The composition of edema fluid and lymph in edema and elephantiasis resulting from lymphatic obstruction. *Am. J Physiol.* 1934 109 572.
30. Eisenman, A. J., A note on the Van Slyke method for the determination of chlorides in blood and tissue. *J Biol. Chem.* 1929 82, 411.
31. Fick, R. A., *Handbuch der Anatomie und Mechanik der Gelenke unter Berücksichtigung der bewegenden Muskeln.* Jena, 1904-11 3 volumes. Cited by Mayeda.
32. Field, M. E., and Drinker C. K., The permeability of the capillaries of the dog to protein. *Am. J Physiol.* 1931 97 40
33. Field M E., Leigh, O C., Jr., Helm, J W., and Drinker C. K., The protein content and osmotic pressure of blood serum and lymph from various sources in the dog *Am. J Physiol.* 1934-35 110 174
34. In Findlay A., *Practical Physical Chemistry* Longmans, Green & Company, New York, 1929
35. Fisher A. G T *Chronic (Non-Tuberculous) Arthritis.* Macmillan Company New York, 1929
36. Fiske C. H., A method for the estimation of total base in urine. *J Biol. Chem.* 1922, 51 55
37. Fiske, C. H., and Litarczek, G Unpublished data.
38. Fiske C. H., and Logan M. A., In *Folin's Laboratory Manual of Biological Chemistry Determination of Calcium.* Appleton-Century Co., New York, 1934 5th ed., p. 349
39. Fiske, C. H., and Logan, M. A., In *Folin's Laboratory Manual of Biological Chemistry Determination of Magnesium in Urine.* Appleton Century Co New York, 1934 5th ed. p. 237
40. Fiske, C. H and Subbarow Y., The colorimetric determination of phosphorus *J Biol. Chem.* 1925 66, 375
41. Folin, O., Two revised copper methods for blood sugar determination. *J Biol. Chem.* 1929 82, 83.
42. Folin, O., and Wu, H., A system of blood analysis. *J Biol. Chem.* 1919 38, 81
43. Forkner C. E., The synovial fluid in health and disease with special reference to arthritis. *J Lab. and Clin. Med.* 1930 15, 1187
44. Fremont Smith, F., Thomas, G W., Dailey M. E., and Carroll, M P., The equilibrium between cerebrospinal fluid and blood plasma. V The osmotic pressure (freezing point depression) of human cerebrospinal fluid and blood-serum. *Brain*, 1931 54 303
45. Frerichs, F. T., in Wagner R., *Handwörterbuch der Physiologie, mit Rücksicht auf physiologische Pathologie.* 1846 III Abt. 1 Synovia, p. 463.
46. Frey H., *Compendium of Histology* Putnam, New York, 1876. (Translated by George R. Cutter.)
47. Friedemann, T E., Cotonio M., and Shaffer P A., The determination of lactic acid. *J Biol. Chem.* 1927 73, 335
48. Gilligan, D. R. Volk, M C., and Blumgart, H. L., Observations on the chemical and physical relation between blood serum and body fluids II. The chemical relation between serum and edema fluids as compared with that between serum and cerebrospinal fluid. *New England J Med.* 1934 210 896.

86. Lieboff S. L., and Kahn, B. S., A rapid and accurate method for the determination of urea in the blood. *J Biol. Chem.*, 1929, 83 347
87. Loeb R. F., Atchley D. W., and Palmer, W. W. On the equilibrium condition between blood serum and serous cavity fluids. *J Gen. Physiol.*, 1922, 4, 591.
88. Maly R., Ueber die Aenderung der Reaction (in der Lösung eines Salzgemesches) durch Diffusion und die dadurch mögliche Erklärung beim Vorgange der Secretion von saurem Harn aus alkalischem Blute. *Berichte der Chem. Gesellschaft zu Berlin*, 1876 9 164
89. Man, E. B., and Peters, J. P., Permeability of capillaries to plasma lipoids. *J Clin. Invest.*, 1933 12, 1031
90. Mayeda, T., Experimentelle histologische Studie über die Synovialmembran. *Mitt. a. d. Med. Fakult. d. k. Univ. zu Tokyo* 1919-20 23 393
91. McEwen, C., Cytologic studies on rheumatic fever. II Cells of rheumatic exudates. *J Clin. Invest.*, 1935 14 190.
92. McLean F. C., and Hastings A. B., The state of calcium in the fluids of the body. I. The conditions affecting the ionization of calcium. *J Biol. Chem.*, 1935 108, 285
93. Meyer A. W. *Lehrbuch der Anatomie*. 1861. Cited by Mayeda.
94. Moore, N. S., and Van Slyke D. D., The relations between plasma specific gravity plasma protein content and edema in nephritis. *J Clin. Invest.*, 1929-30 8, 337
95. Mumtwyler E., Way C. T. and Pomerene E., A comparison of the chloride and bicarbonate concentrations between plasma and spinal fluid and plasma and ascitic fluid in reference to the Donnan equilibrium. *J Biol. Chem.*, 1931 92 733
96. Myers W. K. Keefer C. S., and Holmes W. F., Jr., The characteristics of synovial fluid in gonococcal arthritis. *J Clin. Invest.*, 1934 13 767
97. Ogston, A., On articular cartilage. *J Anat. and Physiol.* 1875 10 49
98. Peters, J. P., *Body Water* Thomas, Springfield, 1935
99. Rainey G. London, Edinburgh and Dublin Philosph. Magazine. On the Anatomy and Physiology of the Vascular Fringes in Joints and Tendons. 1846 Volume 29 Cited by Hammar
100. Rauber A. A., *Lehrbuch der Anatomie des Menschen*. Leipzig 1908. Cited by Mayeda.
101. Rourke, M. D. On the determination of the sodium content of small amounts of serum or heparinized plasma by the iodometric method. *J Biol. Chem.*, 1928 78 337
102. Schmidt, C. L. A., and Greenberg D. M., Occurrence, transport and regulation of calcium, magnesium and phosphorus in the animal organism. *Physiol. Rev.*, 1935 15 297
103. Schneidmuhl G., Beitrag zum femeren Bau der Gelenke bei den grosseren Hausthuieren, speciell des Kniegelenks beim Pferde. *Arch. f. wissenschaft. u. prakt. Thierh.*, 1884, 10 40.
104. Schneider J. Untersuchungen über die Viskosität menschlicher Synovia. *Biochem. Ztschr.*, 1925 160, 325
105. Schrant, J. M., Der Ursprung des Colloids nach dem Holländischen von C. E. Weber, Separatabdruck s. e. Cited by Hammar
106. Seellger P., Ein Beitrag zur pathologischen Physiologie der Gelenke unter Berücksichtigung der Gelenknausbildung. *Arch. f. klin. Chir.*, 1926 142, 606
107. Soubbotine, M., Recherches histologiques sur la structure des membranes synoviales. *Arch. de Physiol. norm. et path.*, 2, 1880 7 532.
108. Stoddard, J. L., and Drury P. E., A titration method for blood fat. *J Biol. Chem.*, 1929 84 741
109. Talbott J. H., and Michelsen, J., Heat cramps. A clinical and chemical study. *J Clin. Invest.*, 1933 12, 533
110. Tillmanns H., Beiträge zur Histologie der Gelenke. *Arch. f. mikr. Anat.*, 1874 10 401
111. Thompson, W. O. Thompson, P. K., and Dailey M. E., The effect of posture upon the composition and volume of the blood in man. *J Clin. Invest.* 1927-28, 5 573
112. Todd R. B. and Bowman, W., *Physiological Anatomy and Physiology of Man*. Longmans London, 1856 I p 126
113. Van Slyke, D. D., Hastings, A. B., Hiller A., and Sendroy J., Jr., Studies of gas and electrolyte equilibria in blood. XIV The amounts of alkali bound by serum albumin and globulin. *J Biol. Chem.*, 1928 79 769
114. Van Slyke, D. D., and Neill J. M., The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. *J Biol. Chem.*, 1924 61 523
115. Van Slyke, D. D., Wu, H., and McLean F. C., Studies of gas and electrolyte equilibria in the blood. V Factors controlling the electrolyte and water distribution in the blood. *J Biol. Chem.*, 1923 16, 765
116. Vaubel, E., The form and function of synovial cells in tissue cultures. II The production of mucin. *J Exper. Med.* 1933 58 85
117. von Holst, G., Serosamucum, eine Mucinsubstanz in Ascitesflüssigkeit und Synovia. *Ztschr. f. physiol. Chem.*, 1904 43 145
118. Walker A. M., Quantitative studies of the composition of glomerular urine. X. The concentration of inorganic phosphate in glomerular urine from frogs and nectum determined by an ultramicro-modification of the Bell Dowsy method. *J Biol. Chem.*, 1933 101 239
119. Walker A. M., Comparison of the chemical composition of aqueous humor, cerebrospinal fluid lymph and blood from

A SIMPLE METHOD FOR DETERMINING THE SYSTOLIC BLOOD PRESSURE OF THE UNANESTHETIZED RAT¹

By J. R. WILLIAMS JR., T. R. HARRISON AND A. GROLLMAN

(From the Department of Medicine Vanderbilt University School of Medicine Nashville and the Department of Pharmacology Johns Hopkins Medical School Baltimore)

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Despite the importance of the determination of blood pressure in the experimental laboratory animals, most of the methods in common use are unsatisfactory. Many of the procedures are applicable only to the anesthetized animal and hence give results which deviate from those observed in the normal animal. Various indirect methods which do not demand the sacrifice of the animal have been described but these usually involve the use of complicated and costly apparatus and are often fraught with grave errors. The difficulty of determining the blood pressure in the rat has excluded the use of this species in many studies for which it offers ideal advantages over the larger mammals. As part of our studies on experimental hypertension we have devised a simple, cheaply constructed apparatus by the use of which it is possible to determine easily and rapidly the systolic blood pressure of the unanesthetized rat. Determinations on as many as one hundred animals may be made in a single day without difficulty.

APPARATUS²

The apparatus consists of a warming box, a suitable holder for the rat, a compression cuff and a plethysmograph. The rat holder is made of a piece of thin walled brass tubing (*A*) (see Figure 1), 6 centimeters in diameter and 10 centimeters long. One end of this is open and the other closed except for a round hole (*B*) 2 centimeters in diameter, through which to bring the rat's tail. This brass cylinder fits into a copper sleeve (*C*) of similar length one end of which is closed with copper screening. If serial

pressures are to be taken on the same rat the outer jacket is wound with a heating coil (*D*) and connected to a rheostat (*E*). We have used the unit from a flat-iron. A thermometer (*F*) is placed near the closed end of the outer tube, which is covered with gauze bandage to permit even distribution of heat.

The blood pressure cuff (*G*) is a modification of that used by Diaz and Levy (1). It is a brass tube 4 centimeters in diameter and 4 centimeters long. A 1.8 centimeter hole (*H*) is made in the proximal end and a 1.5 centimeter hole (*I*) in the distal end. A flange (*J*) is built around these to facilitate the placing of the rubber tubing as shown in the insert in Figure 1. Thin walled soft rubber tubing (*K*) one centimeter in diameter, such as that used for surgical drainage, is then threaded through the holes and carefully fitted over the flanges so as to produce a smooth round aperture. The tubing is held in place by tightly fitting metal inserts (*L* and *M*), as shown in Figure 1. A side connection leads through a T tube to a mercury manometer (*N*) and a sphygmomanometer bulb and release valve (*O*).

The plethysmograph (*P*) is made of 2-centimeter brass tubing 13 centimeters long the ends being constructed the same as the distal end of the pressure cuff. The same kind of rubber tubing used in the pressure cuff is used in the plethysmograph. In putting this in place one must be careful it is not stretched, for 5 centimeters of water pressure must hold it against the tail. The two cuffs are then fastened together as shown in Figure 1. A side connection leads through a short piece of pressure tubing to a three way tap (*Q*), which is connected to a 20 cubic centimeter syringe (*R*) and a water manometer (*S*) made of 1/8th inch glass tubing. The rubber connection should be as short as possible. The manometer, syringe, and plethysmograph are then filled with water.

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² We are indebted to Mr. M. Herblin, Director of the Apparatus Shop, Vanderbilt Medical School, for technical assistance in the designing and construction of this apparatus.

one need not heat the rat holder. If repeated pressures are desired however, one can maintain the necessary vasodilatation by keeping the holder at approximately 37°C . On removal from the warming box the rat is placed in the holder and the tail is inserted through the hole into the cuff and the plethysmograph. Gentle pressure is then applied through the syringe. The pressure cuff is inflated above the systolic level, the syringe is released, and the three way tap turned to connect the water manometer and the plethysmograph. Five to 15 centimeters of water pressure are sufficient to compress the recording cuff snugly around the tail. Air is now slowly released until the water level begins to rise. Under proper conditions this point represents systolic blood pressure. If the rat is properly heated neither restlessness nor sweating will occur, and when the reading is taken there will be a sharp increase in the rate of rise in the water manometer before the mercury manometer has been lowered 10 millimeters below the systolic level. The water level should rise at least a centimeter during this period and should continue rising as the cuff is deflated until the pressure is almost off, when a drop occurs. Successive readings should yield pressures in the same range. A steady rise or fall in successive readings indicates the rat is not properly heated. If one raises the rectal temperature to 40°C , accurate readings will usually be obtained. If the above criteria are used it is not necessary to measure the temperature.

One of the more frequent causes of error in the method is overheating of the animals which causes restlessness and false high blood pressure readings. If the rats are heated to the point of collapse, false low blood pressure readings are obtained. On the other hand, false low readings result from insufficient heating. However, this can be avoided by making sure the rises of the water manometer fulfill the aforementioned criteria and having successive pressures fall within the same range. Air pockets in the plethysmograph make readings difficult. Poorly trained rats will not give consistent pressures. The blood pressure cuff should have at least 3 centimeters of rubber in contact with the tail. Less than this may result in false pressure readings. Another common source of error is having the rubber tub-

ing placed improperly in the plethysmograph. It should be smooth and even and under no tension. If all these criteria are properly fulfilled, accurate blood pressures on rats are readily obtained.

RESULTS

The systolic blood pressures of a group of young adult rats as determined daily for six days (after a period of preliminary training to habituate the animals to the apparatus) are reproduced in Table I. The values obtained illustrate

TABLE I
The blood pressure of the normal rat as determined on five successive days

Rat number	Blood pressure on 5 successive days (mm. Hg)				
	1	2	3	4	5
1	120	115	120	110	120
2	100	110	110	105	110
3	115	125	110	115	120
4	100	110	110	100	105
5	110	115	120	120	115

the excellent day-to-day reproducibility of the values when precautions are taken to avoid excitement, overheating, etc., and when the animals have become accustomed to the procedure. The values of Table I are of the same order of magnitude as those observed by us on numerous animals by direct manometric measurement with a cannula inserted into the aorta in rats anesthetized with nembutal. The values are also in good agreement with those reported by Durant (4) and Leiter (5), using direct manometric measurement. They are also in fair agreement but show much less variation than those reported by Griffith *et al* (6) and Bonsmann (7) using indirect procedures. Woodbury and Hamilton (8) on the other hand, report values of 145 and 187, respectively on two rats as measured by the optical manometer.

We have also applied the apparatus for prolonged studies of the blood pressure on rats in which hypertension has been induced. Typical results showing the high degree of reproducibility of the results obtained on such animals are given in Table II. The determinations were all ways made at the same hour of the day and the animals maintained on a constant diet.

The values reported by Silfverskiöld (9) for

RESPONSES OF NORMAL SUBJECTS AND OF PATIENTS WITH DIABETES INSIPIDUS TO WATER AND SALT INGESTION¹

By H. L. WHITE AND THOMAS FINDLEY Jr.

(From the Departments of Physiology and of Medicine Washington University School of Medicine St. Louis)

(Received for publication March 10 1939)

This paper reports a comparison between normal subjects and patients with diabetes insipidus of some blood and urine responses to the ingestion of water and of hypertonic salt solution, both with and without pitressin. One aspect of this work, the responses of normal and diabetes insipidus subjects to water without pitressin, has already been reported (1). Since the earlier work showed failure of the human diabetes insipidus subject to respond to water ingestion with an acute increase in urine volume one purpose of the present work is to determine whether such subjects show a corresponding failure, as compared with the normal, of responses as regards salt. Ability to conserve salt when on a low salt intake has also been studied. There has further been a comparison of the acute responses to salt ingestion while on an unrestricted and on a low salt intake.

METHODS

The usual procedure has been to get several consecutive 20 or 30-minute urine collections, with 1 or 2 blood samples during this control period. The subject then drank water or salt solution, urine and blood collections being continued. In the experiments with pitressin, a dose of 0.15 unit per kilo subcutaneously was given at the end of the control period 1 hour before water or salt ingestion. Experiments were usually begun early in the morning after a light breakfast or none; no food was taken during the experiments. Urine chlorides were done by a modified alkaline Harvey titration (2). Serum protein nitrogen was determined by a micro-Kjeldahl method, serum chlorides by Sendroy's titrimetric method (3), in many cases serum viscosity as an index of protein and conductivity as an index of chlorides also were determined as previously described (4), with good agreement with the chemical findings.

RESULTS

The acute responses to water ingestion without pitressin by the normal and the diabetes insipidus subject have already been reported (1), the patient with diabetes insipidus differs from the normal in that no acute increase in urine volume occurs. This finding has also been obtained in 6 additional experiments on 2 more cases seen since the first paper. It occurs whether the subject is on unrestricted (average of 10 grams NaCl daily) or on low (1 gram NaCl or less daily) salt intake.

When a normal subject drinks 1200 to 1500 cc. of water one hour after receiving 0.15 unit of pitressin per kilo subcutaneously there is a fall of 12 to 21 mgm per cent in serum NaCl, of about 0.1 gram per cent in serum protein, and no consistent change in rate of chloride output or in urine volume (Figure 1). There have been 7 such experiments on 3 normal subjects. In 2 experiments the rates of chloride output and urine volume have shown the progressive fall seen in control experiments where nothing was given to the subject for several hours except small amounts of water.

When this procedure is carried out on the subject with diabetes insipidus the results are the same as with the normal, except, of course, that urine volume is greatly decreased below its initial level by the pitressin, while in the normal it is unchanged. In neither the normal nor the diabetes insipidus subject is serum chloride or rate of chloride output consistently changed by pitressin, in both the normal and the diabetes insipidus subject the course of the serum chloride and of the rate of renal output of chloride following the ingestion of water is the same with pitressin as without. Figure 2 shows an observation on a subject with diabetes insipidus, there are 8 such observations on 3 cases. If the subject has been for some time on an unrestricted salt intake the

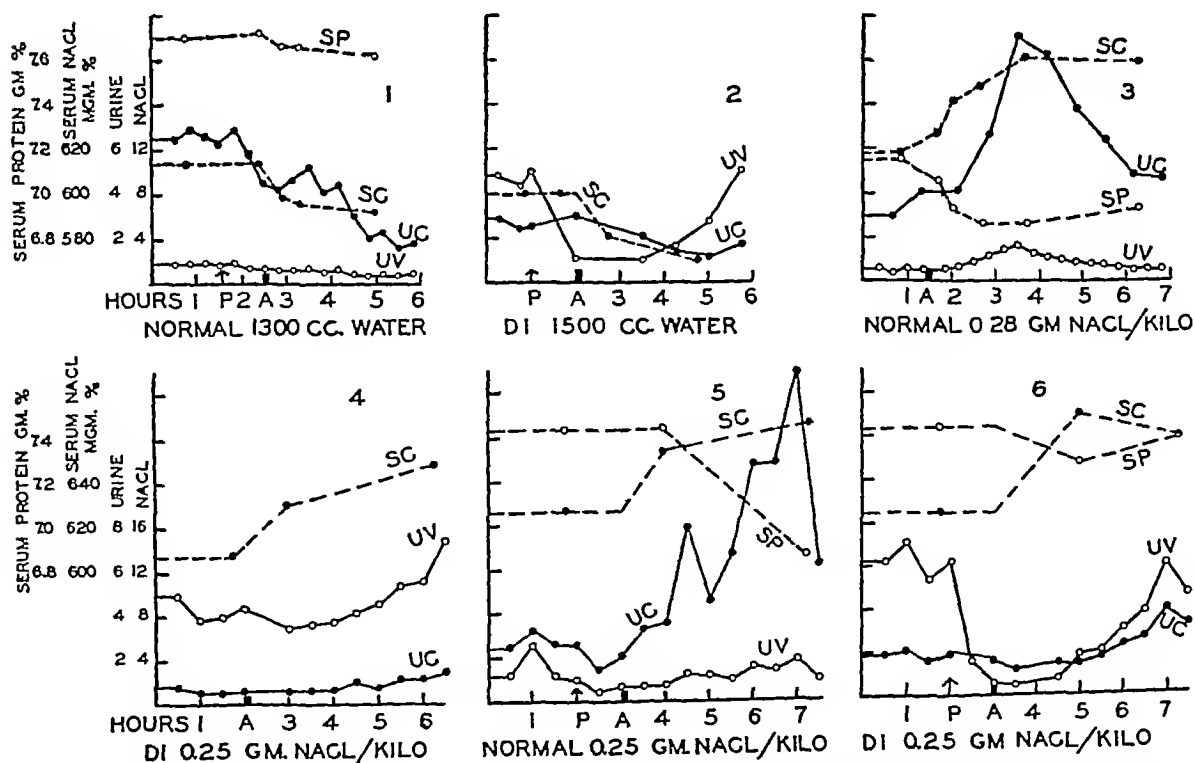
¹ Aided by a grant from the Commonwealth Fund to H. L. White.

initial rate of output may be 3 to 12 mgm of NaCl per minute, while on low intake it may be only a few tenths of a milligram per minute. In either case the rate of output is not consistently changed by pitressin or by water ingestion 1 hour later.

When a normal subject on unrestricted salt intake takes 0.25 gram of NaCl per kilo of body weight in 10 per cent solution, one sees a rise in serum NaCl of from 40 to 60 mgm per cent, a fall in serum protein of from 0.32 to 0.50 gram per cent, and an increase of 200 to 600 per cent in rate of chloride output within the next few hours (Figure 3). When the subject has been on a low salt intake (1 gram daily) for a week to 10 days preceding the experiment, the same results are obtained, except that the percentage increase in rate of chloride output is greater. Thus, in the first condition, the output rises from 3 to 12 mgm of NaCl per minute to 10 to 30 mgm per minute, while in the second case it rises from 0.3 to 0.6 mgm per minute to 5 to 15 mgm per minute.

The fall in serum NaCl is only slight after a 10-day period of low salt intake following unrestricted intake, *e.g.*, fall from 626 to 618 mgm per cent, in a subject able to conserve salt, *i.e.*, one whose renal NaCl output will fall to 0.4 to 0.8 gram daily on a daily intake of 1 gram. Since such a subject is not significantly depleted of salt by such a period of low salt intake, he responds to a large dose of salt in essentially the normal fashion, although the absolute rate of salt output following a given dose is usually less than when such dose is given during a period of unrestricted intake. The total time required for the elimination of such a dose is usually 4 or 5 days when on a daily low salt intake as compared with 3 or 4 days when on unrestricted intake.

In the normal subject, the increase in rate of salt output following such a dose begins in the third or fourth half hour. Many experiments have been carried out in which the urinary response to various doses of NaCl in 10 per cent solution has been followed in 6 normal subjects on unrestricted



FIGS 1 TO 6. At A the subject drank the material designated under each figure, at P 0.15 unit of pitressin per kilo was administered subcutaneously. Urine designates urine volume (UV) in cc. per minute, NaCl designates renal output of NaCl in mgm. per minute (UC), Serum NaCl designates level of serum NaCl in mgm. per 100 cc. (SC), Serum protein designates serum protein in grams per 100 cc. (SP). DI = Diabetes insipidus.

daily salt intake (7 to 14 grams daily) It was found in control experiments, where the subjects took nothing except occasional small drinks of water, that the rate of chloride output usually falls during the day as from 6 to 12 mgm per minute in the morning to 2 to 5 mgm per minute in the afternoon. If, then, after 6 half hourly urine collections beginning at 8 a.m. a dose of salt is taken and the collections continued for several hours more with the result that the rate of salt output rises during the afternoon, this rise may safely be ascribed to the salt ingestion. Many such experiments have led us to conclude that with a dose of 0.17 gram of NaCl per kilo the rate of chloride output usually shows an increase of 50 to 100 per cent, beginning in the fourth or fifth half hour, in the remaining cases the rate at least stays constant i.e. the effect of the salt dose is seen in that the fall of control experiments does not occur. This is about the threshold dose which can safely be predicted to show an appreciable acute effect on rate of chloride output. With 0.22 gram of NaCl per kilo some increase in rate of output has always been seen this is also true with 0.25 and 0.31 gram per kilo the magnitude of the effect being in general proportional to the dose. Also the larger the dose the sooner is the increase in output manifest thus, with 0.31 gram per kilo it is seen in the third half hour.

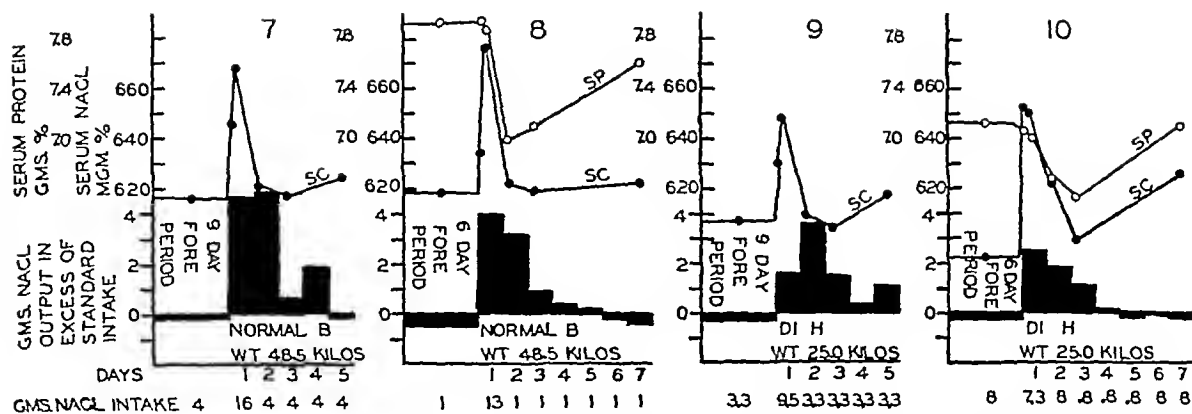
When a subject with diabetes insipidus on unrestricted salt intake took 0.25 gram of NaCl per kilo there was usually no increase in rate of chloride output within the next 5 hours in 3 of the 4 cases studied (Figure 4), 2 of these cases were also studied during a period of low salt intake with the same result. This is not due to a failure of absorption of the salt, since the serum chloride rose as much in these cases as in the normal subjects. One of these cases was the most severe of the 4 a 10-year-old girl weighing 25 kilos with a daily urine output of 9 to 10 liters on 3.3 grams daily salt intake and of 6 liters on 0.8 gram daily (subject of Figure 4). In 1 other case the urinary chloride response has not been essentially different from the normal either on unrestricted or on low salt intake this was a relatively mild case, an adult with a daily urine output of 4 to 5 liters. Various observers (5, 6, 7, 8, 9, 10, 11, 12, 13) have reported that chloride elimination may be delayed in diabetes insipidus

but in only a few cases has the blood been followed or the urine course of the first few hours observed.

The longer time relations of the urinary responses to such a salt dose may be compared in the normal and the diabetes insipidus subject. We have found no significant difference, in both, the excess salt is eliminated in 3 to 4 days when on an unrestricted salt intake and in 4 to 5 days when on low salt intake. We confirm Veil's statement (14) that in normal subjects following a dose of about 0.25 gram of NaCl per kilo the serum chloride is up for the first 24 hours only, being back to normal at the end of 24 hours and thereafter. We do not confirm his finding that the chloride output is up for the first 24 hours, back to normal for the second 24 hours and then up again for the third day, during which the remainder of the dose is eliminated. We find rather that the output for the second 24 hours is almost as great as for the first, it then falls during the third and fourth days at the end of which the dose has been essentially completely eliminated. The striking finding is that during the second 24 hours the output is about as high as during the first 24 hours, although the serum chloride is usually back to normal and sometimes lower. This is true for both the normal and the diabetes insipidus subject. It is also true when the subjects are on low (except for the dose) daily salt intake, the only difference here being that a day more may be required for the dose to be eliminated. A series of observations is shown in Figures 7 to 10.

The stimulus for the maintained increase in output after serum chloride has returned to normal may be an increase in plasma volume, although the mechanism through which this might operate is not clear. Our only clue as to such changes in plasma volume is the plasma protein level this usually but not always is below normal while the excess salt is being eliminated. Even in the cases where plasma protein returns to normal before elimination of the salt dose is completed it may be that plasma volume remains above normal. Within 2 or 3 days diluted plasma protein may be built up to normal by new protein formation even though it is probably safe to regard acute (measured in hours instead of days) falls in plasma protein as indicating increases in plasma volume.

Another interpretation of the maintained increase in salt output after serum chloride has re-



FIGS 7 TO 10 The solid blocks show the amount of NaCl put out in the urine in excess (or deficit) of the standard intake, where standard intake is the daily intake other than the experimental dose. Thus, in Figure 7, the intake in the food was 4 grams daily but on Day 1 there was an additional dose of 12 grams. The sum of the blocks above the zero line adds up to the dose by the time chloride equilibrium is reattained. At equilibrium, daily renal output of NaCl is a few tenths of a gram less than daily intake, as is seen in the foreperiod.

turned to normal is that those elements of the tubular epithelium responsible for chloride reabsorption may, after having once attained salt equilibrium at a higher level, become desalted only slowly even in the presence of an environmental fluid which is again normal with respect to salt, so long as these elements are more than normally salty they will fail to reabsorb salt to the normal degree of completion. The principle is analogous to that in which respiration is believed to be regulated by the state of the interior of the respiratory center cells or peripheral chemoreceptors rather than by the state of the blood *per se*. Whatever be the interpretation, one must believe that the factors regulating salt reabsorption by the tubules are represented by some stimulus acting in or on the tubular cells themselves, it is inconceivable that these cells can be apprised of the existence of salt stores at some distant point in the body except through the operation of some process acting upon them immediately. The acute rise in salt output occurring in normal and in some diabetes insipidus subjects following a large dose of salt is logically explained as due to the accompanying acute rise in plasma salt. The failure of some subjects with diabetes insipidus to show such increase in output in spite of the usual rise in serum chloride may be "explained" by the statement that the tubular cells of such subjects are slower to respond to environmental changes than the normal individual. The maintenance of an increased salt output in both the normal and the diabetes insipidus subject

after the serum chloride has returned to or below normal and the cessation of an increased salt output without further demonstrable change in serum chloride level, when the excess has been eliminated, demands some more subtle mechanism.

When a normal subject takes 0.25 gram of NaCl per kilo 1 hour after 0.15 unit of pitressin per kilo subcutaneously the increases in serum chloride and in chloride output are the same or somewhat less than without pitressin. Four such experiments have been done on 3 normal subjects. Where the rise in serum chloride and in chloride output is less than without pitressin the diminution in effect is presumably due to interference by the pitressin with intestinal absorption. There has been no evidence that pitressin accelerates the elimination of such a dose of salt under our experimental conditions. A typical experiment is shown in Figure 5.

When a subject with diabetes insipidus takes 0.25 gram of NaCl per kilo in 10 per cent solution 1 hour after 0.15 unit of pitressin per kilo subcutaneously we find, as in the normal, that the course is not greatly changed by the pitressin, the rise in serum chloride and in chloride output is the same or somewhat less than without pitressin. Three such experiments have been done on 2 with diabetes insipidus, one is shown in Figure 6 (same subject as in Figure 4). In several other experiments, where the blood was not studied, carried out on another diabetes insipidus subject who failed to show within 5 hours any increase in

chloride output following such a dose of salt with out pitressin, there was still no increase in output following the salt when pitressin was given, i.e., the addition of pitressin did not render normal the response of these subjects to the salt dose.

Some incidental observations may be noted. One is that in the 2 diabetes insipidus cases adequately observed there have been greater spontaneous fluctuations, on constant diet, in the serum chloride level than is usually seen in normal subjects. Thus 1 subject showed within a week high and low values of 631 and 592 mgm of NaCl per 100 cc. respectively, another showed conductivity readings indicating a spread of 41 mgm. per cent. This increased lability of the serum chloride level in diabetes insipidus has also been noted by Marx (15). There has been no correlation between daily variations in serum chloride level and in urine output. One of us (H. L. W.) has observed correspondingly large spontaneous fluctuations in serum chloride level in dogs with experimental diabetes insipidus on constant diet, also without correlation with daily urine output. In our experience, normal subjects (either human or dog) do not show changes in serum chloride as great as 40 mgm per cent even when there are large changes in chloride intake. Thus, a normal child showed within a 5-week period serum NaCl values of 616, 626, 618, and 622 mgm per cent; the first 2 values were obtained during a period of relatively high (4 grams daily) salt intake, the second 2 values during a period of low (1.0 gram daily) intake.

The finding that a large drink of water when preceded or accompanied by pitressin dilutes the serum chloride to as great an extent as it does without pitressin with no increase in amount of urinary chloride output speaks against the view that pitressin 'mobilizes' chloride from tissues into blood. We also fail to find any consistent increase in chloride output or in serum chloride following pitressin unaccompanied by a drink of water.

DISCUSSION

The views expressed here are at variance with those of various German workers. For example, Veil (13, 16) emphasizes the classification of diabetes insipidus into 2 types, the hyperchloremic hypochloruric and the hypochloremic hyper-

chloremic. A partial summary of Veil's views is attempted in the following paragraph.

If a hyperchloremic-hypochloruric case is given 10 to 20 grams of NaCl, his plasma protein does not dilute, as does the normal, rather, it may increase. This means that no water is drawn into the blood and indicates a reduction of the water depots. Since it occurs with normal water content of the blood, it must mean a disturbance of the exchange processes between blood and tissues. Such a case also shows a much greater rise of serum chloride to a given dose of salt than does the normal; the ability of the tissues to take up salt is diminished. In the normal the salt passes from the blood into the tissues as well as into the urine; in diabetes insipidus it fails to enter the tissues. The high molecular concentration of the blood so produced stimulates the kidneys to increased water elimination. Such subjects are much more sensitive to water deprivation than the normal; their plasma protein rises much more on deprivation. This is not true with primary polydipsia or with hypochloremic hyperchloremic diabetes insipidus. The subject with hyperchloremic diabetes insipidus always shows a retarded elimination of chloride; this is not due to any altered behavior of the kidneys but to a disturbance of the blood tissue salt exchange. Pituitrin acts on the tissues in general, not on the kidneys, restoring to normal the tissues' ability to hold salt. It therefore has an antidiuretic action on the hyperchloremic but not the hypochloremic type of diabetes insipidus because the latter type already shows normal ability of the tissues to hold salt.

The evidence for the statements in the preceding paragraph is scattered through a number of publications and in our opinion, is not always adequate. First as to the idea that the hyperchloremic type of diabetes insipidus is merely a disturbance of exchange between tissues and blood, the fact that the blood salt rises on salt administration without corresponding rise in renal salt output shows that the kidneys are not responding normally. Second, it is not true in our experience, that the serum chloride of the diabetes insipidus subject rises higher than in the normal in response to a given dose of salt. Third it is not true that the serum protein of a diabetes insipidus subject shows less dilution than the normal in response to a given dose of salt. In the ex-

periment of Figure 4 the serum protein was not followed, but in other experiments it has shown a dilution on salt ingestion quite comparable with the normal. Thus, in a pair of experiments on a normal subject and on the diabetes insipidus subject of Figure 4 the serum protein in grams per 100 cc in the normal was 7.82 before the salt dose of 0.25 gram per kilo, 7.96 1 hour after the dose, 7.88 4 hours after the dose, 6.96 24 hours later, and 7.10 48 hours later, while the corresponding figures for the diabetes insipidus subject were 7.12, 7.06, 6.99, 6.64, and 6.53. Finally, we cannot understand why an increased molecular concentration of the blood should stimulate the kidneys to increased water elimination in the absence of any increase in elimination of solids.

We have found no consistent differences in the blood responses of the diabetes insipidus subject as compared with the normal on ingestion of water or of salt, either with or without accompanying pitressin administration. We have not had any cases fail to show an antidiuretic response to pitressin, as Veil says is the case with the hypochloremic type, although we recognize that this does not mean that such cases cannot exist. It is our impression that Veil's hypochloremic cases were not true diabetes insipidus.

Our concept of this condition, as contrasted with that of various of the German workers, follows. Diabetes insipidus is due to a deficiency of the antidiuretic principle secreted by the *pars nervosa* of the hypophysis and adjacent floor of the third ventricle. This principle acts on the renal tubules rather than on the tissues in general, it enables the tubules to reabsorb water from a hypertonic solution in their lumen. The renal tubules in diabetes insipidus are less sensitive than normally to acute changes in water or salt concentration of the blood. There is no need to assume a disturbance of exchange of water and salt between tissues and blood in this condition, the blood responses to salt and to water ingestion are normal. There is no basis for the division into hyperchloremic and hypochloremic types, one and the same case while on constant diet may show alternately a hyperchloremia and a hypochloremia. True diabetes insipidus can be distinguished from primary or psychogenic polydipsia by withholding water for 6 to 12 hours: if the urine flow falls to normal and its concentration rises to normal the

case is a primary polydipsia. If the polyuria persists, the case is either diabetes insipidus or belongs to a type of renal disease unable to put out a concentrated urine, the latter condition should be recognizable by other appropriate measures. One such measure may be mentioned. If the patient is put on a low salt intake (1 to 2 grams daily) (in the absence of gross sweating or diarrhea) he will, if a case of diabetes insipidus, come into chloride equilibrium within 2 to 4 days, without a consistently significant lowering of serum chloride, *i.e.*, he will behave in these respects as does a normal subject. A case of renal disease of the type likely to be confused with diabetes insipidus will, on the other hand, usually show a diminished ability to conserve salt as well as water, he will continue in negative chloride balance and, if the procedure is prolonged, will show a progressive fall in serum chloride.

SUMMARY

With 4 cases of diabetes insipidus studied, all have failed to show within a few hours an increase in urine flow in response to a large drink of water, when urine flow preceding the drink was below maximal.

Normal subjects show within 2 hours a greatly increased rate of urinary chloride output in response to a dose of 0.25 gram of NaCl per kilo in 10 per cent solution. Of the 4 cases of diabetes insipidus studied, 3 have failed to show any increase in chloride output within 5 hours while 1 has responded essentially normally. Where a delay in chloride output occurs, the delay is not due to a failure of rise of serum chloride.

Both normal and diabetes insipidus subjects excrete about 40 per cent of the above dose of salt during the first 24 hours, about 40 per cent during the second 24 hours, and the remainder within the next day or two. This is true whether the experiment be done during a period of high or low daily salt intake, provided the subject is able to conserve salt adequately, *i.e.*, to come into salt equilibrium on low salt intake before serum salt is significantly lowered. The only qualification is that a day longer may be required for elimination of the dose on a low as compared with a high daily salt intake. The serum chloride is high for the first day but is practically always down to or below

normal by the end of 24 hours and thereafter. The maintenance of the high rate of chloride output during the second 24 hours is not satisfactorily explained, although some guesses are proposed.

In neither normal nor diabetes insipidus subjects has 0.15 unit of pitressin per kilo subcutaneously resulted in any consistent increase in rate of urinary chloride output, either with or without a large drink of water, the behavior of the serum chloride is not affected by the pitressin. The antidiuretic action of this dose of pitressin is marked for 4 to 6 hours in both normal and diabetes insipidus subjects, following thus it diminishes rapidly and is usually not apparent after 8 hours. The antidiuretic action of pitressin in the normal subject is, of course, observed by following the pitressin with a large drink of water.

The rise in serum chloride and in rate of chloride output following a dose of 0.25 gram of NaCl per kilo in 10 per cent solution is either unaffected or somewhat diminished by a dose of 0.15 unit pitressin per kilo subcutaneously 1 hour previously in both the normal and the diabetes insipidus subject.

Since the blood changes in all of the procedures carried out have been the same in the normal and in the diabetes insipidus subject, differences in urinary responses may logically be referred to differences in sensitivity of the kidneys, presumably the tubules, to environmental changes. Each of the 4 cases of diabetes insipidus studied has shown kidneys less sensitive than the normal to the fall in plasma molecular concentration brought on by a large drink of water. Three of the 4 cases have shown kidneys less sensitive (within the first few hours) than the normal to the increase in plasma molecular concentration brought on by a large dose of NaCl (one (the least severe) has shown essentially normal sensitivity in this respect).

It is believed that there is no justification for the division of cases with diabetes insipidus into hyperchloremic hypochloruric and hypochloremic hyperchloremic types or for the view that the condition is primarily a disturbance of the processes of exchange of water and salt between tissues and blood.

We wish to express our thanks to Dr. A. F. Hartmann for the facilities of the metabolism ward of the St. Louis Children's Hospital in the study of 1 case with diabetes insipidus and 1 normal case.

BIBLIOGRAPHY

- 1 Findley T., Jr., and White, H. L., The response of normal individuals and patients with diabetes insipidus to the ingestion of water. *J. Clin. Invest.*, 1937, 16, 197.
- 2 Peters, J. P., and Van Slyke, D. D. *Quantitative Clinical Chemistry Vol II Methods*. Williams and Wilkins Co., Baltimore, 1932 p. 833.
- 3 Sendroy J., Jr. Microdetermination of chloride in biological fluids, with solid silver iodate. II. Titrimetric analysis. *J. Biol. Chem.*, 1937, 120, 405.
- 4 White, H. L., and Findley T., Jr., Time relations in renal excretion of threshold and no-threshold substances. *Am. J. Physiol.*, 1937, 119, 740.
- 5 Allen, F. M., and Sherrill, J. W., Diet treatment of diabetes insipidus. *J. Metab. Res.*, 1923, 3, 479.
- 6 Ambard, L., La sécrétion chlorurée dans le diabète insipide. *Médecine*, 1931, 12, 230.
- 7 Decourt, J., Meyer L., Audry M., and Lesourd, R., Diabète insipide. Action du régime déchlorure sur le polyurie. *Bull. et mém. soc. méd. d. hop. de Paris*, 1934, 50, 1695.
- 8 Dreyfus, G., Remarques sur la physiopathologie du diabète insipide. *Bull. et mém. soc. méd. d. hop. de Paris*, 1934, 50, 1755.
- 9 Lichtwitz, L., Die Konzentrationsarbeit der Niere. *Arch. f. exper. Path. u. Pharmacol.*, 1911, 65, 128.
- 10 Pellegrini, G., Contributo allo studio del diabete insipido. I I rapporti fra il ricambio idrico intermedio e la poliuria. *Clin. med. ital.*, 1931, 62, 899.
- 11 Rabinowitch, I. M., Metabolic studies on a case of diabetes insipidus. *Arch. Int. Med.*, 1921, 28, 355.
- 12 Rosenbloom, J., and Price, H. T., Metabolism study of a case of diabetes insipidus. *Am. J. Dis. Child.*, 1916, 12, 53.
- 13 Veil, W. H., Über intermediäre Vorgänge beim Diabetes insipidus und ihre Bedeutung für die Kenntnis vom Wesen dieses Leidens. *Biochem. Ztschr.*, 1918, 91, 317.
- 14 Veil W. H. Über die Bedeutung intermediärer Veränderungen im Chlorstoffwechsel beim Normalen und beim Nierenkranken. *Biochem. Ztschr.*, 1918, 91, 257.
- 15 Marx, H., Der Wasserhaushalt des gesunden und kranken Menschen. Springer Berlin, 1935 p. 267.
- 16 Veil, W. H., Physiologie und Pathologie des Wasserhaushaltes. *Ergeb. d. inn. Med. u. Kinderh.*, 1923, 23, 648.

LACTIC ACID PRODUCTION DURING REST AND AFTER EXERCISE IN SUBJECTS WITH VARIOUS TYPES OF HEART DISEASE WITH SPECIAL REFERENCE TO CONGENITAL HEART DISEASE¹

By PHILLIP HALLOCK

(From the Division of Internal Medicine University of Minnesota Hospitals Minneapolis)

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The defects found in congenital heart disease are frequently of such character as to disturb the normal pathways of circulation through the heart. The common types of congenital lesions responsible for diverting the normal course of blood are interventricular septal defects with or without pulmonary stenosis, auricular septal defects and patent ductus arteriosus. In these anomalies the hemodynamic relationship between the right and left side of the heart becomes so altered as to lead to a mechanical interference with the proper oxygenation of the blood. When this obtains, quantities of blood pass unacrated (venous arterial shunt) from the right to the left side of the heart giving rise to varying degrees of oxygen unsaturation cyanosis, and anoxemia. On the other hand the course of the blood stream may be shunted from the left to the right side of the heart (arterial venous shunt), thereby permitting oxygenated blood to pass back into the lesser circulation. Under the latter conditions there may be neither anoxemia nor cyanosis.

In this study we have sought to obtain data regarding the presence or absence of anoxemia and factors influencing its development. Information of this kind is of course important in congenital heart affections because prognoses, particularly of the potentially more serious defects, depend in general on the degree of anoxemia and the extent of the increase of the work of the heart. The degree of cyanosis is a valuable guide, but is not an accurate measure of anoxemia. If polycythemia is present there may be a sufficient quantity of oxygen for the tissues and in addition enough reduced hemoglobin to cause cyanosis. Conversely anoxemia may be present without visible cyanosis. We have attempted here to investigate this problem by studying the variations

in blood lactic acid levels at rest and following slight exercise. The amount of lactic acid in the blood can be utilized as a measure of anoxemia since it is recognized that a reduction in the oxygen supply to the tissues can, by inhibiting the oxidative recovery process, prevent the combustion of lactic acid and its conversion to glucose, thus giving rise to the accumulation of this substance in the tissues and blood stream (1, 2).

METHOD

The investigation was carried out in the morning with the subjects in the fasting state. The patients rested at least thirty minutes before being placed in the chair ergometer and a sample of blood was drawn from the anterior cubital vein. With the exception of a few who were too ill all subjects were subjected to a mild exercise test. The exercise on the chair-ergometer is simple and easily executed after the subject has been given a few simple instructions. Briefly the apparatus consists of a comfortable chair projecting out from which is a double set of small tracks. The feet are fastened to two runners which in turn fit on the tracks. In the act of extension of the legs a set of weights, connected to the heels of the runners by a combination of bicycle chains are raised a given distance. The work performed is calculated in terms of kilogrammeters. An average of about 1500 kgm. m. of work was done in ten minutes. We have considered this amount of physical work as equivalent to mild exercise. Several patients, however were unable to carry out this amount of exercise because of fatigue and dyspnea. After exercise the subject remained sitting quietly in the chair. Seven minutes after the cessation of exercise a second sample of blood was removed from the anterior cubital vein. Lactic acid reaches a maximum concentration in the anterior cubital vein seven minutes after the termination of work on the chair-ergometer (3).

The samples of blood were then analyzed for lactic acid in duplicate or triplicate by the method of Freidemann Cotonio and Shaffer (4). The error involved in the method is no greater than 1 mgm. per cent.

MATERIAL

Thirty nine persons were studied. In several instances the individual experiments were re-

¹ This study was carried out in the Medical Unit of Wilhelmina Hospital, University of Amsterdam, and the Cardiac Department of London Hospital, London.

peated Of the total number tested eighteen were patients with congenital heart disease (Tables II and III) with clinical or radioscopic evidence of either intra or extra cardiac communications between the venous and arterial circulation In this group eight were diagnosed as having interventricular septal defects, seven cases showed the classical findings of patent ductus arteriosus, two cases with permanent cyanosis were diagnosed as tetralogy of Fallot, and one case possessed either an interventricular septal defect or a patent auricular septal defect The diagnosis in each of the eighteen cases of congenital heart disease was based on the auscultatory findings, radioscopic appearance of the heart, and electrocardiographic evidence In two cases it was necessary to rely solely on the auscultatory findings because of the absence of enlargement of one or more chambers of the heart and the absence of enlargement of the pulmonary artery In these two cases, however, the auscultatory findings were quite conclusive in respect to the location and character of the murmur and of the accompanying localized thrill

Ten of the subjects studied had normal cardiovascular systems and their ages ranged from ten to fifty-nine years There were six cases of acquired valvular defects, two of which were associated with auricular fibrillation Of the remaining five cases there were two of coronary thrombosis, two of hypertension associated with coronary disease, and one of ventricular tachycardia

RESULTS

The concentration of lactic acid in venous blood of the normal human at rest with the Freidemann, Cotonio, and Shaffer method (4) varies from 10 to 20 mgm per 100 cc of blood (5) Table I shows the lactic acid values obtained from ten normal subjects at rest and after the above described mild exercise After these same individuals were submitted to our exercise test the concentration of venous blood lactate never exceeded 21 mgm per 100 cc. The average concentration of lactic acid at the resting level was 13.8 mgm per 100 cc while after mild exercise it was 16.3 mgm, an average increase of slightly more than 2 mgm per 100 cc No patient showed nor complained of fatigue or dyspnea after the exer-

TABLE I
Lactic acid content of venous blood during rest and after exercise in normal subjects

Case	Age	Sex	Amount of work performed	Lactic acid	
				Before	After
	<i>years</i>		<i>kgm m</i>	<i>mgm per 100 cc</i>	<i>mgm per 100 cc</i>
1	22	F	1,260	12.8	15.0
2	23	M	1,440	10.3	12.9
3	11	F	1,050	13.6	16.6
4	19	F	1,287	11.4	13.7
5	13	M	1,350	15.0	17.0
6	24	M	1,920	15.8	16.4
7	13	F	1,092	12.6	16.8
8	59	M	2,000	15.3	20.5
9	10	F	350	12.0	17.2
10	24	M	1,700	15.4	15.8
11	35	M	1,500	17.7	17.8
Average				13.8	16.3

cise and all stated they could carry on without any difficulty

The resting lactic acid levels and those following mild exercise for the cases with venous arterial shunts are tabulated in Table II These patients are classified under the cyanotic group because of permanent cyanosis or its development after exercise (transient type) The changes in the concentration of the lactic acid following exercise are obviously marked, with the exception of Case 16 In every other instance there is more than a 65 per cent increase Two separate experiments were carried out on Case 16 and the results were substantially in agreement, showing a 9 per cent increase from the resting value after the exercise test

A fairly definite relationship exists between the lactic acid concentration and dyspnea, the patients with the highest lactic acid levels showed the greatest amount of dyspnea after exercise (See Table II)

Twelve cases comprise the acyanotic group (Table III) None showed visible cyanosis before or after mild exercise The highest lactic acid concentration after exercise was found in Case 21 In this group the height of concentration of lactic acid after exercise was again related to the degree of dyspnea In those cases in which the lactic acid response was within the normal range, dyspnea was neither observed nor noted by the patient This table further indicates that the blood lactic acid values may rise well above the

TABLE II

Cyanotic group Lactic acid levels of venous blood before and after standard exercise in those cases of congenital heart disease associated with venous-arterial shunts

Case	Age	Sex	Diagnosis	Work performed	Lactic acid		Hemoglobin*	Red blood cells	Remarks
					Before	After			
	years			kgm. m.	mm per cent	mm per cent	per cent	millions	
11	63	M	Interventricular septal defect	1200	18.44	43.25	121	7.5	Cyanotic, became dyspneic after this exercise and cyanosis more intense
12	19	M	Tetralogy of Fallot	340	17.98	40.94	106	11.0	Continuous cyanosis since birth became extremely dyspneic and blue after this amount of exercise
13	17	F	Tetralogy of Fallot	1350	16.03	28.44	132	7.5	Cyanotic all her life. Cyanosis increased after work. Developed slight dyspnea after exercise
14	23	F	Patent ductus	875	13.20	28.68	90	5.0	Slight degree of cyanosis. Cyanosis did not increase after work. Slightly dyspneic and fatigued after exercise
14	23	F	Patent ductus	984	13.30	27.54			As above
15	21	F	Interventricular septal defect associated with patent ductus arteriosus	1720	20.88	47.10	82	5.0	No cyanosis but became cyanotic after exercise. Complained of fatigue. Moderate degree of dyspnea
15	21	F	Interventricular septal defect associated with patent ductus arteriosus	1475	18.48	29.56			As above
16	25	M	Interventricular septal defect with patent ductus	1512	20.78	22.01	130	7.0	Slight to moderate cyanosis. Not fatigued nor dyspneic after exercise. Cyanosis more marked after exercise
16	25	M	Interventricular septal defect with patent ductus	1800	18.85	22.57			As above
				Average	17.58	32.00			

* 17 grams of hemoglobin per 100 cc. of blood is equivalent to 100 per cent hemoglobin.

upper range of normal (Cases 17, 18, 21, 22, 23) without the development of cyanosis. Thus the abnormal rise of lactic acid is not necessarily associated with cyanosis.

In Table IV are shown the lactic acid values in chronic valvular defects with and without auricular fibrillation. In the two cases of mitral stenosis associated with auricular fibrillation (Cases 31 and 32) there was a definitely abnormal rise in the lactic acid values following exercise. In Case 31

a slight amount of cyanosis developed but in Case 32 this phenomenon was absent yet both developed dyspnea after the exercise test. In Case 29, likewise, an abnormal lactic acid rise was observed and even in the absence of auricular fibrillation, dyspnea developed after 1500 kgm m of work. In the remaining cases no abnormal response was obtained. In Case 35 a slightly elevated resting lactic acid value was obtained.

Several interesting findings are brought out in

TABLE III
*Acyanotic group Lactic acid levels of venous blood before and after standard exercise
 in those cases of congenital heart disease associated with arterial-venous shunt*

Case	Age	Sex	Diagnosis	Work performed	Lactic acid		Remarks
					Before	After	
	<i>years</i>			<i>kgm m</i>	<i>mgm per cent</i>	<i>mgm per cent</i>	
17	32	M	Probably case of interven- tricular septal defect	1175	22 89	28 34	Some dyspnea after exercise
18	21	M	Patent ductus	2100	15 80	23 20	No dyspnea No fatigue
19	31	F	Patent ductus	1275	19 65	21 73	Slight dyspnea after work Short of breath on exertion all life
20	32	F	Patent ductus	750	13 34	15 19	Only complained of feeling tired No dyspnea
21	28	F	Patent ductus	1250	15 44	37 87	Moderately dyspneic Breath- lessness since able to walk
22	22	M	Interventricular septal de- fect	1510	15 71	26 70	Slight dyspnea
23	47	M	Interventricular septal de- fect	2000	21 20	31 73	Slight dyspnea
24	12	F	Interventricular septal de- fect	1095	11 88	13 00	No dyspnea
25	5	F	Probably interventricular septal defect	120	13 54	14 36	No dyspnea
26	6	F	Probably interventricular septal defect	240	14 17	14 77	No dyspnea
27	10	M	Interventricular septal de- fect	1460	16 84	20 59	No dyspnea
				Average	16 40	24 31	
28	23	M	Interventricular septal de- fect	1000	28 64	53 90	Marked dyspnea (See Table V, Group VII)

Table V Because of the poor condition of some of these patients it was impossible to submit them to the exercise. Cases 36 and 37 were elderly subjects with acute coronary closure but without apparent signs of congestive failure. Both showed moderately high lactic acid values at rest. It is very likely that the above cases would have developed dyspnea upon exertion. Case 38 had auricular fibrillation in addition to hypertension and coronary arteriosclerosis. His resting lactic acid level was at the upper limit of normal. Following exercise it rose to 30 mgm and moderate dyspnea developed. Case 39 showed a high resting lactic acid value, 37 mgm. This was a case of congestive failure resulting from paroxysmal ventricular tachycardia of long duration. This patient was dyspneic and orthopneic.

DISCUSSION

Effect of cardiac shunts on blood lactic acid

If anoxemia can develop as a result of the shunting of venous blood from the right heart to the left through unaerated channels (veno-arterial) we should expect to find a corresponding increase in the blood lactic acid level following exercise. On the other hand, if the shunt is arterial-venous, then no rise of the blood lactic acid level would be expected. The findings in Table II showing the effect of exercise on the venous blood lactic acid level at rest and following exercise are striking in that they indicate that anoxic anoxemia (6) can occur without cardiac failure. The most obvious explanation of this situation is that adequate amounts of unaerated blood are shunted into the

TABLE IV

Lactic acid values of venous blood before and after standard exercise in cardiac disorders resulting from rheumatic valvular defects with and without auricular fibrillation

Case	Age	Sex	Diagnosis	Work performed	Lactic acid		Remarks
					Before	After	
29	years 29	M	Mitral stenosis and aorta in sufficiency	1500 kgm. m.	19.22 mgm. per cent	28.22 mgm. per cent	Slight dyspnea after test
30	16	M	Mitral insufficiency with stenosis	1920	11.13	13.00	No dyspnea nor fatigue
31	45	M	Mitral stenosis with auricular fibrillation	718.5	21.63	23.93	Slight cyanosis, fatigue and slight dyspnea after test
32	43	M	Mitral stenosis with auricular fibrillation	1575	16.01	25.17	Slightly fatigued and dyspneic after test
33	29	M	Aortic insufficiency	840	14.87	16.00	Severe headache. Refused to carry on with work
34	21	M	Aortic insufficiency	1900	13.61	15.88	No dyspnea nor fatigue
				Average	16.08	20.36	

TABLE V

Lactic acid values of venous blood before and after standard exercise in other cardiac conditions

Case	Age	Sex	Diagnosis	Work performed	Lactic acid		Remarks
					Before	After	
	years			kgm. m.	mgm. per cent	mgm. per cent	
CORONARY THROMBOSIS GROUP V							
35	60	M	Coronary thrombosis with diabetes	No work	23.42		Occlusion six days old
36	59	M	Coronary thrombosis with hypertension	No work	19.83		No signs of congestive failure angina pectoris for past seven years
				Average	21.67		
HYPERTENSION WITH CORONARY ARTERIOSCLEROSIS, GROUP VI							
37	50	M	Hypertension with coronary arteriosclerosis	No work	29.14		No signs of congestive failure
38	60	F	As above with auricular fibrillation	1 013	19.83	30.05	Dyspnea following exercise
				Average	24.40	30.05	
CONGESTIVE FAILURE GROUP VII							
39	50	M	Ventricular paroxysmal tachycardia	No work	37.72		No cyanosis, pulse 170
28	23	M	Interventricular septal defect	1000	28.64	53.90	Marked dyspnea after exercise
				Average	33.18	53.90	

general circulation, thus creating an oxygen deficit (anoxemia). If, following the exercise test the recovery phase be prolonged because of this oxygen shortage, an excessive accumulation of lactic acid will be expected in the venous blood for some time after the termination of the exercise. The amount of unaerated blood shifted will depend on the size of the defect, and the height of blood pressure in the two circuits. As long as the pressure in the right heart or pulmonary artery is maintained higher than on the left side unaerated blood will pass through the defect.

In congenital heart disease, tissue anoxia is apparently not present during rest for the lactic acid values are essentially normal. While the circulation rate may be slowed somewhat if polycythemia is present, enough oxygen seems to be available to remove the lactic acid which is normally formed at rest.

When Table II (cyanotic group) is compared to Table III (acyanotic group) it will be noted that the lactic acid concentration, particularly after exercise, is with only a few exceptions consistently higher in the cyanotic group. The presence of cyanosis, though not directly responsible for higher blood lactic acid levels, indicates a potential state of oxygen deficiency which easily can be precipitated by mild physical exertion. It is a common clinical observation that in congenital heart defects with venous-arterial shunts, cyanosis becomes more intense upon such slight exertion as walking. This observation was made in the cyanotic cases following the exercise test. This is to be attributed to the fact, as pointed out by Haldane (7), that in morbus caeruleus after exercise, the blood returned from the musculature is extremely poor in oxygen. Furthermore, Lunds-gaard and Van Slyke (8) point out that as much as one-third of the venous blood entering the right heart chamber must be shunted over directly into the systemic circulation before the cyanotic threshold is exceeded. In extreme cases of cyanosis as much as two-thirds of the venous stream may be shunted, thus leading to a high degree of oxygen-unsaturation. It is reasonable to assume therefore that cyanosis in congenital heart disease indicates the presence and approximate extent of the shunt, though not in quantitative terms.

When polycythemia is present in congenital heart disease it appears to be a compensatory process arising from the stimulation of erythropoietic

bone marrow tissue by anoxia. As a consequence the red cell count increases and therefore the capacity of the blood to carry oxygen is increased. In spite of its apparent beneficial effects, its presence indicates, as our studies show, that the liability to tissue anoxia is great even following mild exertion (Table II).

The degree of cyanosis is not necessarily an index of the amount of oxygen lack that will develop following muscular work. Case 16 (Table II) illustrates this point. Two separate sets of determinations were carried out on this individual, the results being in very good agreement. The resting values on both occasions were slightly elevated as compared with the normal group. Following exercise the average rise of lactic acid was about 2 mgm per 100 cc of blood. This patient had continuous cyanosis and following the exercise the cyanosis deepened appreciably, yet the lactic acid rise was only slight. Marked secondary polycythemia was present in this subject, and there was moderate cardiac enlargement. We must conclude from this case that tissue anoxia, at least to any marked degree, may be absent even in the presence of cyanosis and polycythemia. This patient complained of no fatigue after exercise and dyspnea was not noted. He stated he could easily carry on the work and was the only patient in this group who did not show evidence of fatigue or dyspnea. Obviously there was enough reduced hemoglobin to produce cyanosis, yet there was a sufficient amount of oxygen to prevent a muscle oxygen debt.

Blood lactic acid in acquired heart disease

When the five uncomplicated cases of mitral stenosis and aortic insufficiency (Table IV) are considered, it will be noted that the lactic acid levels before and after exercise are within normal limits and that the exercise response is the same as in normal subjects. On the other hand, the two cases of mitral stenosis associated with auricular fibrillation showed a moderate rise above the normal lactic acid level. Also, slight to moderate dyspnea was noted after the exercise. It would appear that in cases of valvular defects associated with auricular fibrillation a mild state of anoxia develops following exercise. In this group can also be included Case 38 with hypertension, auricular fibrillation, and evidence of marked en-

largement of the left ventricle. Dyspnea followed the exercise test and, while the resting level of lactic acid was at the upper limits of normal the concentration after exercise rose to 30.05 mgm. The lactic acid rise in this case was undoubtedly consequent to left ventricular failure.

Of the two cases of coronary thrombosis studied, Table V Group V neither had any signs of congestive failure, still the resting blood lactic acid levels were slightly elevated.

Two cases of congestive failure were studied Table V, Group VII. Both showed high blood lactic acids levels at rest. Case 28 was given the standard exercise test and the blood lactic acid rose to 53 mgm per 100 cc. of blood. This was the highest value recorded in the series, indicating the great tendency to tissue anoxia when the cardiac reserve is impaired.

That the concentration of lactic acid may reach abnormally high concentrations even at rest in cases of circulatory failure was demonstrated by Meakins and Long (9) and confirmed by Jervell (10). They pointed out, furthermore that the accumulation of lactic acid in the blood was in proportion to the severity of the circulatory failure and was excessively great after exercise. The results in our two cases were in entire accord with the findings of the above investigators.

The increase in the concentration of lactic acid following even mild exercise in cardiac failure must be attributed to a deficient oxygen supply to the tissues. With mild physical exertion the oxygen supply cannot be increased in proportion to the increased oxygen requirement and a greater accumulation of lactic acid in the blood is found.

Relation of blood lactate values to cardiac insufficiency

From the studies above it is apparent that the cause of hyperlactacidemia in acquired heart disease is myocardial insufficiency. It is then necessary to inquire whether the high lactate values obtained in Table II (cyanotic type of congenital heart disease) and Table III (acyanotic type) can be accounted for on the basis of myocardial weakness. In order to obtain data in this direction we submitted the patients with congenital heart disease to radiocardiological and electrocardiographic examination of the heart the purpose being not only to determine the type of enlargement for diagnostic purposes but also to

determine the degree of enlargement from a viewpoint of cardiac efficiency. The degree of enlargement is a fair index of the severity of the myocardial lesion and of the liability to failure (11, 12, 13). When Table VI is examined all the post-exercise values are abnormally high except in Case 16. Case 16 showed moderate enlargement of all chambers of the heart, yet the lactate concentration remains about stationary. Certainly from the point of view of enlargement the lactate values after exercise in this instance should be high if myocardial insufficiency were present. On the other hand Cases 11, 12, and 13 showed a very slight degree of enlargement with no evidence of myocardial disease by the electrocardiogram. Yet the lactate values doubled after exercise. From a consideration of these observations it would be inconsistent to attribute the high lactate values to myocardial insufficiency. In Cases 14 and 15 only are we justified in attributing the high lactate value to myocardial insufficiency. Yet in Case 14 gross failure was absent, for the venous pressure was 9 cm H₂O.

When Table III (acyanotic group of congenital heart disease) is examined, abnormally high values are found in Cases 17, 18, 21, 22, and 23. Case 17 showed only slight enlargement. Case 18 showed no enlargement. Case 21 revealed a large pulmonary artery with very slight if any enlargement of the right ventricle. Case 22 showed no enlargement of the ventricles except slight enlargement in the region of the conus and Case 23 showed no enlargement. Of course, it is possible that temporary myocardial insufficiency could have manifested itself during exercise.

The above studies demonstrate that two factors may operate either singly or in combination to increase the lactic acid concentration of the blood in congenital heart disease. These are myocardial insufficiency and the presence of either intra or extra cardiac shunts. In the presence of cardiac enlargement, to at least a moderate degree, the cardiac reserve may be impaired and an increase in the blood lactate may be found after exercise. The co-existence of a veno-arterial shunt would heighten the blood lactate value. However, when cardiac enlargement is minimal or absent then the high lactate values may be preponderantly a direct result of the veno-arterial shunt. On the other hand the elevation of the blood lactate in some cases of the acyanotic group (arteriovenous

TABLE VI
Significant cardiac findings in the congenital heart groups

Case	Diagnosis	Lactic acid		Radiocardiological findings	Electrocardiogram findings	Blood pressure	Auscultatory findings
		Before	After				
		mgm per cent	mgm per cent			mm Hg	
CYANOTIC GROUP							
11	Interventricular septal defect	18 44	43 25	Generalized enlargement slight Rt Vent ↑ Lt Vent ↓ Pul Art ↓	Normal rhythm Low voltage Lead I P1 bifid P2 P3 prominent L A D	135/80	Systolic murmur all areas Loudest over pulmonic area
12	Tetralogy of Fallot	17 98	40 94	Rt Vent ↑ Lt Vent ↓ Pul Art normal Conus ↓	Normal rhythm T1 T2 prominent and upright T3 inverted P tendency to bifid all leads R.A D	138/85	Rough, blowing systolic murmur over pulmonic area, but no thrill 2d pulmonic absent
13	Tetralogy of Fallot	16 00	28 44	Dextrorotation of aorta Pul Art not enlarged Conus of Rt Vent ↑ and slight generalized enlargement	Normal rhythm R.A.D P3 and T3 flat	135/82	Harsh, rough systolic murmur and systolic thrill over pulmonary area 2d pulmonic sound absent
14	Patent ductus arteriosus	13 20	28 68	Pul Art ↑↑ Conus ↑↑ Rt Vent ↓ Lt Vent ↓ Venous pressure 9 cm H ₂ O	Normal rhythm L A D P.R. interval = 0 26 sec	128/65	Machinery-like murmur heard maximum over pulmonic area 2d pulmonic sound present
15	Interventricular septal defect associated with patent ductus arteriosus	20 88	47 10	Pul Art ↑↑ Rt Vent ↑↑ Conus ↑↑ Lt Vent ↓	Notching of P waves in Leads I and II Otherwise normal Normal rhythm	125/85	Systolic murmur heard over 3d interspace just to left of sternum
16	Patent ductus arteriosus associated with interventricular septal defect	20 78	22 01	Pul Art ↑↑ Lt Vent ↓ Conus ↑↑ Rt Vent ↑↑ Venous pressure 13 cm H ₂ O	R.A.D High P2 T2 Increased amplitude of Q R S complexes Normal rhythm	130/82	2d pulmonic sound markedly accentuated Systolic murmur heard best over 3d interspace to left of sternum preceded by snapping 1st sound and followed by snapping 2d sound followed by soft blowing diastolic murmur
ACYANOTIC GROUP							
17	Probable case of interventricular septal defect	22 89	28 34	Pul Art sl enlargement Rt Vent ↓ Conus ↑	Normal rhythm T2 T3 Inverted Rt A D	100/60	Basal systolic thrill pulmonary region Systolic murmur all areas, maximum over base
18	Patent ductus Botalli	15 00	23 00	No generalized enlargement Pul Art ↑↑ and pulsating	Rt A D Normal rhythm	145/95	Short blowing systolic murmur over apex. Systolic thrill over pulmonary area with systolic murmur 2d pulmonic sound present

TABLE VI—Continued

Case	Diagnosis	Lactic acid		Radiocardiological findings	Electrocardiogram findings	Blood pressure	Auscultatory findings
		Before	After				
		mm per cent	mm per cent			mm. Hg	
ACYANOTIC GROUP (Continued)							
19	Patent ductus arteriosus	19 00	21 00	Pul. Art. †† with pulsation R.V. †† Conus †† Pul. branches †	R.A.D. Normal rhythm	120/90	Systolic thrill and murmur over 1st and 2d left interspaces
20	Patent ductus arteriosus	13 00	15 00	Pul. Art. †† Other changes slight	R.A.D. Normal rhythm	130/80	Machinery like murmur heard over pulmonary area 2d pulmonary sound heard
21	Patent ductus arteriosus	15 44	37.87	Pul. Art. †† with marked pulsation of pulmonary vessels. No aortic window Conus. † Sl. enlargement of rt. vent. only	Bifid P2 P3	110/65	Systolic and diastolic murmur over pulmonary artery and thrill
22	Interventricular septal defect	15 00	26 00	Slight enlargement in conus region	Normal	110/70	Loud rough systolic murmur heard maximum along sternal border with systolic thrill at level of 3d interspace
23	Interventricular septal defect	21 00	31 00	No definite enlargement	Normal	135/80	Loud rough systolic heard best over lower sternum with systolic thrill
24	Interventricular septal defect	11 00	13 00	No definite enlargement	Normal	110/60	Loud rough systolic murmur heard over entire sternum but heard maximum along left sternal border at level of 4th interspace accompanied by systolic thrill
25	Probable case of interventricular septal defect	13 00	14 00	No enlargement	Normal	100/60	Loud rough systolic murmur heard maximum along left sternal border at level of 3d interspace accompanied by systolic thrill
26	Interventricular septal defect	14 00	14 77	Rt. Vent. †† Conus †† Lt. Vent. †	R.A.D.	98/58	Thrill and loud systolic murmur elicited over level of 3d interspace just to left of sternum
27	Interventricular septal defect	16 00	20 00	Rt. Vent. † Conus † Pul. Art. not enlarged	Normal	105/60	Systolic murmur loud rumbling heard best over 3d interspace just to left of sternal borders. Level of 3d interspace
28	Interventricular septal defect Congestive failure	28 64	53 90	Rt. Vent. ††† Conus. †† Lt. Vent. † Pul. Art. † Lt. Aur. not enlarged	R.A.D.	130/78	Systolic murmur loud and harsh heard best along left sternal border at level of 2d interspace Diastolic murmur questionable

†† = Moderate enlargement.
 † = Slight enlargement
 L.A.D. = Left axis deviation
 Rt. Vent. = Right ventricle.

R.A.D. = Right axis deviation.
 ††† = Considerable enlargement.
 Lt. Vent. = Left ventricle
 Pul. Art. = Pulmonary artery

shunts) requires further explanation. The high values cannot be explained on the basis of myocardial insufficiency because it would be extremely difficult to comprehend how a heart normal in size and not showing any evidence of myocardial disease could become temporarily incompetent during mild exercise. It is our impression that the high lactate values obtained are probably due to the shunting of blood from the left to the right side of the heart through large defects and that therefore the output of blood through the aorta into the peripheral circulation does not increase in proportion to the oxygen demand of the muscles in exercise.

From the foregoing survey it is apparent that the hyperlacticacidemia which develops after exertion is constantly associated with the presence of dyspnea, regardless of the cause of anoxemia. When dyspnea is present one can predict the occurrence of abnormally increased amounts of lactic acid in the tissues and blood during exercise. Valentin (14) previously adduced evidence to show that blood lactic acid is high in all conditions involving dyspnea. The observations made in this study indicate that dyspnea invariably accompanies the anoxemic state.

CONCLUSIONS.

1 An increased concentration of lactic acid in the venous blood is evidence of an inadequate supply of oxygen to the tissues.

2 There is only a slight increase of lactic acid in the blood following mild exercise in normal individuals, an average increase of about 2 mgm per 100 cc of blood above the resting value. The normal upper limit of concentration of venous blood lactic acid following our exercise test did not exceed 21 mgm per 100 cc of blood.

3 The blood lactic acid studies show that tissue anoxia is not present at rest in patients with congenital heart disease, either in the presence or absence of cyanosis.

4 Following mild exercise there is a definitely abnormal rise of blood lactic acid in the cyanotic group of congenital heart disease, indicating a greater liability to the development of tissue oxygen deficit after even slight physical exertion.

5 The presence of cyanosis and polycythemia in congenital heart disease does not necessarily

indicate that oxygen deficit will develop following mild exertion for no significant rise of lactic acid level occurred in a case of morbus caeruleus.

6 Following mild exertion, a definitely abnormal rise may occur in some acyanotic cases of congenital heart disease, but the rise is not as great on the average as in the cyanotic group.

7 When dyspnea follows mild exercise the presence of tissue oxygen want may be assumed to be present regardless of what specific cardiac defect is ultimately responsible.

BIBLIOGRAPHY

- 1 (a) Hill, A. V., The mechanism of muscular contraction. *Physiol. Rev.*, 1922, 2, 310
- (b) Hill, A. V., and Long, C. N. H., Muscular exercise, lactic acid and the supply and utilization of oxygen. *Ergebn. d. Physiol.*, 1925, 24, 43
- (c) Hill, A. V., Long, C. N. H., and Lupton, H., Muscular exercise, lactic acid and the supply and utilization of oxygen. Part I Introduction. *Proc. Roy. Soc., s B*, 1924, 96, 438
- 2 Meyerhoff, O., Chemical Dynamics of Life Phenomena. Lippincott, Philadelphia and London, 1924
- 3 Hallock, P., Blood lactic acid after exercise with particular reference to polycythemia rubra vera. *Proc. Soc. Exper. Biol. and Med.*, 1938, 38, 587
- 4 Freidemann, T. E., Cotonio, M., and Shaffer, P. A., The determination of lactic acid. *J. Biol. Chem.*, 1927, 73, 335
- 5 Peters, J. P., Van Slyke, D. D., Quantitative Clinical Chemistry Vol I Interpretations Chapter X. Williams and Wilkins Company, Baltimore, 1931
- 6 Barcroft, J., Anoxemia. *Lancet*, 1920, 2, 485
- 7 Haldane, J. S., Respiration. Yale University Press, New Haven, 1922.
- 8 Lundsgaard, C. and Van Slyke, D. D., Cyanosis Medicine Monographs Williams and Wilkins Co., Baltimore, 1923
- 9 Meakins, J., and Long, C. N. H., Oxygen consumption, oxygen debt and lactic acid in circulatory failure. *J. Clin. Invest.*, 1927, 4, 273
- 10 Jervell, Otto, Investigations of the concentration of lactic acid in blood and urine. *Acta med Scandinav.*, 1928, Supp 24, Chapter III, pp 23 to 26
- 11 Parkinson, J., Enlargement of the heart. Lumleian Lectures. *Lancet*, 1936, 1, 1337 and 1391
- 12 Grant, R. T., After histories for ten years of 1000 men suffering from heart failure. *Heart*, 1933, 16, 275
- 13 Hallock, P., Enlargement of the heart. Its recognition by the radiologic method. *Minnesota Med.*, 1938, 21, 303
- 14 Valentin, F., Über den Milchsäuregehalt des Blutes. *München. med. Wchnschr.*, 1925 72, 86

THE USE OF CO₂ INHALATION AS A TEST OF CIRCULATION TIME¹

By RICHARD GUBNER, SIDNEY SCHNUR, AND J. HAMILTON CRAWFORD

(From the Department of Medicine Long Island College of Medicine and the Kings County Hospital, Brooklyn)

While other functions of the circulatory system have long held the attention of physiologists and clinicians interest in the velocity of blood flow is of more recent date. Numerous investigators have established that alterations in the velocity of blood flow may be of considerable clinical significance in various pathological states such as heart disease hyperthyroidism and various pulmonary conditions.

The clinical inapplicability of fluorescein, introduced by Koch (1), and of radon emanation, with which Blumgart and Weiss (2) performed valuable work on the circulation time in different segments of the vascular system led Weiss and his colleagues to seek other substances which might be used objectively to test the rate of blood flow. Histamine was first suggested (3) the endpoint being an intense flush of the face. This method was discarded however because of the delayed reaction time and the many unfavorable reactions which attended its use. Sodium cyanide, which measures the circulation time from the arm to the carotid sinus was next introduced by Robb and Weiss (4) with respiratory stimulation as the endpoint. This method has achieved some degree of clinical application but its use has not become general because of the alarming reactions of syncope and respiratory arrest which may occasionally occur.

Other more innocuous methods have therefore been introduced. Most of these have a subjective endpoint which detracts from their value. All these substances, as well as cyanide and histamine require intravenous injection and several may produce local thrombosis and pain if extravasation takes place. They are, therefore, not very satisfactory for repeated tests. Many of these agents, in addition, have individually undesirable features. Dangerous ectopic rhythms may occur with the use of calcium salts in digitalized pa-

tients (5). Nausea and vomiting may follow the unpleasant taste induced by saccharin and decholin. Sudden death has been reported with the use of ether (6).

Ether was introduced by Hitzig (7) to distinguish "right" from "left heart time"; ether measures the velocity of blood flow from the antecubital veins to the pulmonary capillaries. The "left heart time" may be estimated indirectly by subtracting the ether time from the values obtained by methods which determine the arm to tongue time. The two injections are performed separately to reduce the incidence of thrombosis which nevertheless frequently occurs. The "left heart time" is of greater clinical interest since this time is predominantly prolonged in left ventricular failure occurring as a result of rheumatic, luetic, hypertensive coronary, or other types of heart disease (2, 3, 8).

A simple direct, and harmless method of determining "left heart time" is available through the inhalation of CO₂. CO₂ inhalation was applied to test circulation time by Bornstein in 1912 (9). In fact this was the first attempt to measure circulation time clinically. He however used only 5 to 7 per cent CO₂ and the long circulation times of 11.5 to 16.5 seconds reported by him as normal values in the few cases he studied may be attributed to the low concentration of CO₂.

TECHNIQUE

A basal metabolism apparatus from which the lime chamber has been removed serves conveniently for the performance of the test. A cylinder of CO₂ is substituted for the oxygen cylinder and sufficient CO₂ is introduced into the gas chamber to give about 4 liters of a 50 per cent mixture of CO₂ and air. After a maximal expiration to the outside the subject takes a rapid full inspiration of the CO₂ mixture through the mouth, which is immediately followed by a second rapid deep respiration to insure adequate diffusion of the gas into the alveoli. The beginning of the first in-

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spiration is considered the start of the test. The endpoint is announced subjectively by a distinct sensation of warmth passing over the head and frequently, by slight transient vertigo, and objectively by a quickening and deepening of respiration (Figure 2) The effect persists for several seconds and disappears completely within one-half minute

The individual sensitivity to CO₂ varies considerably In the normal subject a single inhalation of 50 per cent CO₂ is usually adequate to produce a distinct endpoint, but some subjects require stronger concentrations of CO₂ to produce a response when a single inhalation is used Inspiration of the gas in very high concentrations acts as an irritant to many In patients with heart disease it is frequently difficult to obtain an endpoint with single inhalations of CO₂ because of the impaired respiratory exchange due to decreased vital capacity, and delayed diffusion of gases into the alveoli resulting from decreased lung elasticity, as demonstrated by Siebeck (10) For these reasons two inhalations of 50 per cent CO₂ are routinely employed as described above.

In concentrations much below 50 per cent the endpoint becomes delayed Thus in one subject the endpoint increased from 7 seconds with a 50 per cent mixture, to 9 seconds with a 25 per cent mixture, and to 15 seconds with a 12.5 per cent mixture. In another subject whose circulation time was 5 seconds with a 50 per cent mixture, the endpoint became similarly prolonged to 8 seconds with a 25 per cent mixture, and to 9 seconds with a 12.5 per cent mixture

RESULTS

Values obtained in normal subjects and in pathological cases are graphically tabulated in Figure 1

In performing repeated tests on over thirty normal subjects we found the lung to respiratory center (or possibly carotid sinus) circulation time to be much shorter than that observed by Bornstein The normal CO₂ times ranged from 5 to 10 seconds, the majority being 7 to 8 seconds They were constant within one second in any single individual under similar circumstances

TABLE I
Cyanide, ether, and CO₂ times compared in the same subjects

Patient	Clinical condition	Circulation times			
		Cyanide	Ether	Cyanide ether	CO ₂
		seconds	seconds	seconds	seconds
S	Hypertensive heart disease decompensated	34	20-22	14	18
S	Hypertensive heart disease decompensated	31	18-20	13	16
C	Atrophic arthritis	14	8	6	7
S	Rheumatic heart disease well compensated	17	8	9	8
T	Rheumatic heart disease well compensated	16	6	10	8
A	Rheumatic heart disease well compensated	15	9	6	7
W	Rheumatic heart disease well compensated	15	6	9	7
C	Hypertensive heart disease decompensated	35	20	15	18
L	Rheumatic heart disease decompensated	26 (saccharin)	9	17	16

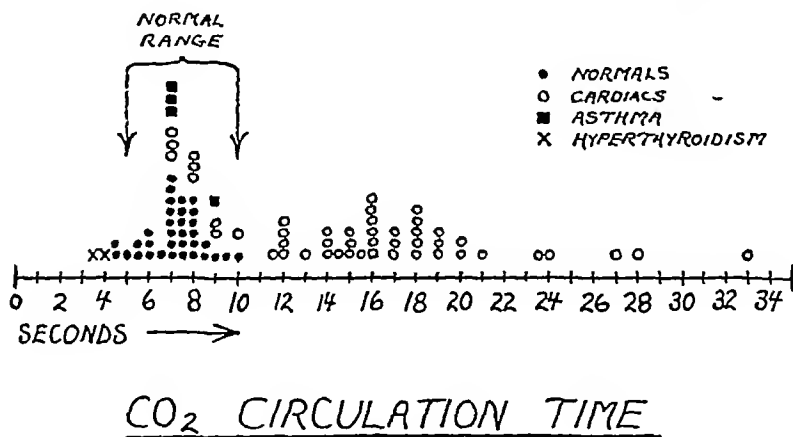


FIG 1 CO₂ CIRCULATION TIME

These values accord well with the normal 'left heart' circulation time obtained indirectly by subtracting right heart time (measured with ether) from combined right and left heart' time (measured with decholin cyanide, saccharin or calcium salts). The latter most often ranges between 12 and 17 seconds whereas 'ether time' averages about 6 seconds with an upper normal limit of 8 seconds. Cyanide ether and CO₂ times were determined in several normal and cardiac subjects. The CO times corresponded almost precisely to the difference between cyanide and ether times in the same subjects (Table I). Stewart *et al* (19) using our method found close agreement between the CO time and the difference between decholin and ether times in four subjects on whom these tests of circulation time were performed.

Figure 2 shows the effect of a single inhalation of 50 per cent CO₂ in a normal subject. Five and five tenths seconds after inspiration simultaneous with a subjective endpoint of warmth in the head and slight vertigo there occurred a sharp stimulation of respiration with a marked increase in volume and rate which subsided completely in less than one minute.

Figure 3 shows the effect in a patient with rheumatic heart disease and pulmonary congestion, whose saccharin and ether times, tested the day before were 26 and 9 seconds respectively. After a few inhalations of 50 per cent CO₂, a subjective endpoint occurred at 16 seconds at the point indicated by the flick in the tracing. This was followed immediately by increased ventilation. The latter was chiefly in rate rather than volume and while definite was not so striking as

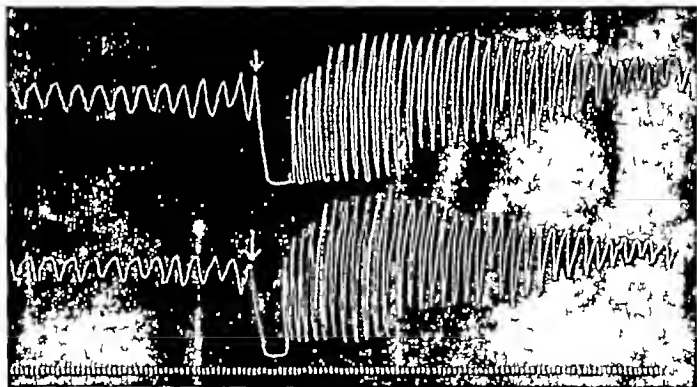


FIG. 2. INHALATION OF 50 PER CENT CO₂ IN A NORMAL SUBJECT. ENDPOINT AT 5.5 SECONDS.

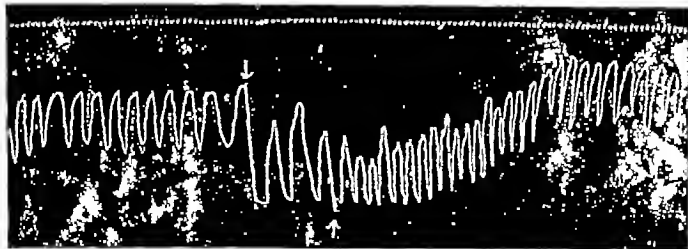


FIG. 3. INHALATION OF 50 PER CENT CO₂ IN A RHEUMATIC CARDIAC. ENDPOINT AT 16 SECONDS.

in the previous tracing of the normal subject. During the period of hyperventilation the chest was held in an inspiratory position (Figure 3), a further indication of the dyspnea which the patient experienced.

Barr and Peters (11) have emphasized that the cardiac patient is unable to increase the volume of his respiration considerably because even at rest breathing is close to the maximal capacity. To quote Barr and Peters, "A normal individual with an original vital capacity of 4000 cc may show under the stimulus of CO_2 a tidal air of 1500 to 2000 cc. In a decompensated cardiac whose vital capacity may be only 1500 cc, the tidal air will not rise above 500 to 750 cc with maximum CO_2 stimulation. The volume of respiration is strictly limited. Any attempt to increase it is accompanied by marked subjective dyspnea."

In left heart failure of whatever cause, CO_2 times have been found distinctly prolonged, commensurate with the degree of decompensation. The longest time observed was 33 seconds. In pulmonary congestion of moderate degree the values obtained have most often been between 15 and 20 seconds. Several patients have been studied while decompensated and during recovery. Thus, one patient who entered in heart failure had a CO_2 time of 24 seconds which fell progressively with improvement to 10 seconds, remaining steady at 11 seconds up to the time of discharge. Re-admitted in failure again two months later, the patient showed a CO_2 time of 22 seconds, which similarly decreased to 11.5 seconds with improvement and rose again to 17 seconds with a temporary clinical relapse. Another patient suffering from hypertensive heart disease and auricular fibrillation with heart failure had a CO_2 time of 18 seconds which fell to 13 seconds the same day after phlebotomy was performed.

Table II summarizes the changes in circulation time during recovery in eight patients who entered the hospital with congestive failure secondary to hypertensive heart disease. In all these cases the CO_2 times were initially prolonged and fell toward normal coincident with improvement. The shortening of the circulation time was closely related to the clinical course.

Normal values of 7 to 10 seconds have been found in well compensated cases of heart disease.

TABLE II
 CO_2 circulation times in patients with heart failure and during recovery (hypertensive heart disease)

Patient	Date	CO_2 time sec onds	Clinical condition
N	1937-1938		
	November 10	24	Decompensated pulmonary edema auricular fibrillation
	November 11	15	Markedly improved
	November 12	10	Improved
P	November 18	11	Out of bed
	November 11	18	Pulmonary edema before phlebotomy
	November 11	13	After phlebotomy
	November 15	14	
McN	March 25	27	Congestive failure basal râles enlarged liver edema
	March 29	28	Unchanged
	March 31	23	Improved râles persist liver slightly enlarged no edema
	April 4	16	Improved few râles liver not felt no edema
	April 8	9	Well out of bed
S	March 29	20	Congestive failure basal râles enlarged liver edema
	March 31	15	Improved basal râles
	April 4	15	Basal râles
	April 8	12	Improved occasional râle
K	March 31	14	Congestive failure auricular fibrillation râles enlarged liver edema
	April 8	10	Out of bed no râles
B	March 31	16	Decompensated basal râles weight 118 lbs
	April 4	15	Basal râles, weight 111 lbs
	April 8	12	Occasional basal râle
J	March 31	18	Decompensated basal râles enlarged liver edema weight 166 lbs
	April 4	14	Improved basal râles weight 145 lbs
	April 8	12	Compensated weight 138 lbs
R	March 31	20	Decompensated basal râles enlarged liver edema
	April 4	15	Basal râles enlarged liver
	April 8	12	Occasional basal râle

In one case with ascites and edema finally diagnosed as portal cirrhosis, in which at first the differential diagnosis from heart disease was difficult the CO_2 time was normal being 7 to 8 seconds. Four asthmatic patients without heart disease had normal CO_2 times, three being 7 seconds and one 9 seconds. This group is of particular interest since the circulation time was normal despite a considerable diminution in vital capacity. The prolonged time in heart disease cannot therefore be attributed to a delayed diffusion of gas into the alveoli, since conditions of impaired respiratory exchange exist in asthma, and the circulation time is nevertheless normal.

An interesting experience was the finding of an unusually rapid CO_2 time of 4 seconds in a patient who had auricular fibrillation with a very rapid ventricular rate. This led to the suspicion of hyperthyroidism, and further history disclosed the development of symptoms of Graves' disease following a severe psychic trauma a few months

before Basal metabolic determinations were repeatedly +60 and at operation a toxic diffuse goiter was found. With clinical improvement and a decrease in metabolism to +12 following thyroidectomy the CO₂ time became prolonged to 9 seconds more than double its original value. Another patient convalescing on a surgical ward from a laceration of the foot, was tested as a supposed normal and was also found to have an unusually rapid CO₂ time of 3.5 seconds. A more careful history and physical examination together with repeatedly high basal metabolic rates also led to a diagnosis of hyperthyroidism previously unsuspected.

DISCUSSION

CO₂ inhalation is apparently entirely harmless being but a temporary exaggeration of a physiological stimulant. There is some question as to whether the stimulation of respiration is due to a direct action of CO₂ on the respiratory center or on the carotid sinus. Although anoxemia has been proven by Heynaus (12) to stimulate respiration solely through the chemical receptors in the carotid sinus CO₂ probably acts directly on the respiratory center as well as on the carotid sinus (13) and such authorities as Haldane and Priestley (14) conclude that the reflex control of breathing by chemical stimulation of the carotid nerve endings has not been established at any rate as regards CO₂. The brief inhalation of concentrated CO₂ does not appear to have any deleterious effect on the circulation. Berencsy (15) found no change in the electrocardiograms of dogs during short periods of respiration of pure CO₂ apart from a transient sinus bradycardia which he attributed to a trigeminovagal reflex. Prolonged respiration of CO₂ as Grollman (16) and others have shown may increase the cardiac output significantly.

Transient vertigo frequently occurs which may be due either to a sudden rise in intracranial pressure or to the anesthetic action of CO₂, as it is an anesthetic in high concentration.

Apart from the subjective dyspnea and vertigo there are other sensations produced by CO₂ inhalation which afford distinct endpoints. Thus there is regularly felt a transient warmth and fullness passing over the head and frequently there is a visible flush of the face. The sensation of warmth

and fullness in the head is probably due to a direct vasodilating action of CO₂ on the cerebral blood vessels. This view is supported by an experiment in which manometric readings were made of spinal fluid pressure during inhalation of CO₂. The patient suffered from cardiac decompensation due to hypertensive heart disease. The initial spinal fluid pressure was 24 cm H₂O. After a single deep inhalation of CO₂ it remained unchanged until 17 seconds later when simultaneous with subjective and objective endpoints the spinal fluid pressure rose several cm of H₂O with each systole to a height of 40 cm at about 40 seconds after which it declined to its original level within one minute. With continuous breathing of 30 per cent CO₂ the spinal fluid pressure similarly began to rise abruptly with the subjective and objective endpoints at 17 to 18 seconds and within 1½ minutes attained 60 cm H₂O at which time the patient experienced marked distress and hyperpnea. Inhalation of CO₂ was discontinued at this time and the pressure fell to its initial level of 24 mm within 2 minutes. A control period of voluntary hyperpnea did not significantly alter the spinal fluid pressure so that the marked rise may be attributed to the vasodilating action of CO₂ with increased cerebral blood flow. Bouckaert and Jourdan (17) Schmidt (18) and several other investigators have likewise demonstrated a direct vasodilating action of CO₂ on the cerebral blood vessels by other methods.

The slight flush of the face which is sometimes observed normally is much more striking in hyperthyroidism. In the two cases of hyperthyroidism already referred to there was an intense flush of the face and a sensation of warmth over the entire body followed by profuse sweating. This reaction disappeared in one patient who had a thyroidectomy with the return of basal metabolism to normal. In another patient with cardiac decompensation of uncertain etiology a marked reaction of this sort gave the first clue of a hyperthyroid state.

SUMMARY

CO₂ inhalation may be employed clinically to estimate circulation time. The CO₂ test measures left heart time (lung to respiratory center). Its advantages are that it is a physiological respiratory stimulant, it is entirely harmless, the effect

is transitory, it does not require injection, and it may be used repeatedly in the same subject

The circulation time, by this method, is prolonged in heart disease commensurate with the degree of left heart failure. Normal values range from 5 to 10 seconds and correspond closely to the expected results according to the cyanide and ether times

BIBLIOGRAPHY

- 1 Koch, E, Die Stromgeschwindigkeit des Blutes. *Deutsches Arch f klin Med*, 1922, 140, 39
- 2 Blumgart, H L, and Weiss, S, Studies on velocity of blood flow. Velocity of blood flow in normal resting individuals, and critique of method used. *J Clin Invest.*, 1927, 4, 15
Studies on velocity of blood flow, pulmonary circulation time in normal resting individuals. *Ibid.*, 1927, 4, 399
- 3 Weiss, S, Robb, G P, and Blumgart, H L, Velocity of blood flow in health and disease as measured by effect of histamine on minute vessels. *Am Heart J*, 1929, 4, 1
- 4 Robb, G P, and Weiss, S, Method for measurement of velocity of pulmonary and peripheral venous flow in man. *Am Heart J*, 1933, 8, 650
- 5 Bower, J O, and Mengle, H A K., Additive effect of calcium and digitalis, warning, with report of two deaths. *J A M A*, 1936, 106, 1151
- 6 Lemoff, H D, Complication following use of saccharin and ether as circulation time test. *J A M A*, 1935, 105, 1759
- 7 Hitzig, W M, Use of ether in measuring circulation time from antecubital veins to pulmonary capillaries. *Am Heart J*, 1935, 10, 1080
- 8 Miller, H R., and Furman, M, Pulmonary blood velocity in congestive heart failure. Velocity in pulmonary venous circuit. *Proc. Soc Exper Biol and Med*, 1935, 32, 728
- 9 Bornstein, A, Über die Messung der Kreislaufzeit in der Klinik. *Verhandl d Deutsch Kongresses f inn Med*, 1912, 29, 457
- 10 Siebeck, R, Die funktionelle Bedeutung der Atemmechanik und die Lungenventilation bei Kardialer Dyspnoe. *Deutsches Arch f klin. Med.*, 1912, 107, 252
- 11 Barr, D P, and Peters, J P, Studies of respiratory mechanism in cardiac dyspnea. III Effective ventilation in cardiac dyspnea. *Am J Physiol*, 1920, 54, 345
- 12 Heymans, C, Bouckaert, J J, and Regniers, P, Le Sinus Carotidien et la Zone Homologue Cardio-Aortique. *Doin, Paris*, 1933, pp 164 to 173
- 13 Gemmill, C L, and Reeves, D L, Effect of anoxemia in normal dogs before and after denervation of carotid sinuses. *Am J Physiol*, 1933, 105, 487
- 14 Haldane, J S, and Priestley, J G, *Respiration*. Clarendon Press, Oxford, 1935, 2d ed, pp 132 and 17
- 15 Berencsy, G, Die Wirkung der Inhalation von CO auf das Elektrokardiogramm der Tiere. *Klin Wchnschr*, 1934, 13, 587
- 16 Grollman, A, *The Cardiac Output of Man in Health and Disease*, C C Thomas, Baltimore, 1932, p 163
- 17 Bouckaert, J J, and Jourdan, F, Recherches sur la physiologie et la pharmacodynamie des vaisseaux cerebraux, influence de l'anhydride carbonique. *Arch. internat. de pharmacodyn. et de therap*, 1936, 54, 155
- 18 Schmidt, C F, Intrinsic regulation of circulation in parietal cortex of cat. *Am J Physiol*, 1936, 114, 572
- 19 Stewart, H J, Heuer, G J, Detrick, J E, Crane, N F, Watson, R. F, and Wheeler, C H, Measurements of the circulation in constrictive pericarditis before and after resection of the pericardium. *J Clin Invest.*, 1938, 17, 581

CLINICAL STUDIES OF THE BLOOD VOLUME VI CHANGES IN BLOOD VOLUME IN PERNICIOUS ANEMIA IN RELATION TO THE HEMATOPOIETIC RESPONSE TO INTRA-MUSCULAR LIVER EXTRACT THERAPY¹

By JOHN G GIBSON 2b

(From the Medical Clinic of the Peter Bent Brigham Hospital and the Department of Medicine Harvard Medical School Boston)

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The blood volume in pernicious anemia has been studied repeatedly by a variety of methods and all authors have commented upon the extremely low level of the total volume of circulating red cells found in the severe stages of this disease. There is less unanimity of opinion as regards the abnormality of both the plasma and total blood volume at various levels of anemia the relationship between the severity of the disease and the degree of hypovolemia and the changes in plasma, red cell, and total blood volume that occur during the course of successful liver therapy. Plasma volume was thought to be normal by Bock (1), Denny (2) and Murphy, *et al* (3) or higher than normal Keith (4), Rusynak (5), and De Wesselow and Bamforth (6). Denny (2) found no relation between the severity of the anemia and the total blood volume level whereas Smith (7) noted that the total amount of hemoglobin was reduced in proportion to the severity of the disease. During treatment with liver the total blood volume was found to increase, due chiefly to a great increase in the red cell volume (6) which may be three or fourfold (3). The plasma volume was found to fall (6) during recovery, or increase (8), or remain fairly constant (3). Höll (8) could not find any relationship between the observed changes in the total blood volume and either the hematocrit or hemoglobin content of the blood but De Wesselow and Bamforth (6) stated that the "increase in percentage of red cell volume and hemoglobin estimations gives an accurate index of the actual increase in red cell substance and hemoglobin during treatment."

¹ This study together with those previously reported in a series of papers entitled "Clinical Studies of the Blood Volume. I to V" inclusive, published in the *Journal of Clinical Investigation*, were aided by a grant from the Proctor Fund for the Study of Chronic Diseases Harvard Medical School.

It is evident that the current conception of the state of the blood volume during the various phases of pernicious anemia and the changes that take place during therapy is confused. It, therefore seemed worth while to study the problem again, employing a dye method for determining the plasma and total blood volume in which the errors inherent in the techniques employed by the authors mentioned above are minimized (9 10).

MATERIAL STUDIED

Six male and 4 female patients, in whom the diagnosis of pernicious anemia was made on the Medical Service or in the Blood Clinic of the Out-Door Department of the Peter Bent Brigham Hospital, were studied before and during the course of treatment with liver extract. All of these patients had hyperchromic anemia, high color index, gastric achlorhydria, and all experienced a marked rise in their reticulocyte count after treatment with liver extract. Initial blood volume studies were made before treatment was begun, at intervals during treatment with liver extract and, in 8 of the 10 cases, when the red blood cell count had reached a level of five million. Three of these patients were started on a single dose of extract² derived from 600, 500, 400 and 75 grams of liver (60 50 40 and 75 U.S.P. units) respectively and 6 on small daily doses of concentrated extract the total amount given in 10 daily doses being derived from 75 grams of liver (75 U.S.P. units) in 4 cases and 100 grams of liver (10 U.S.P. units) in 2 cases. Following the initial treatment, liver extract in amounts derived from 50 to 100 grams (5 to 10

² Extracts used were Lederle "Solution Liver Extract Parenteral." The large single doses were of a preparation containing 10 U.S.P. units in 3 cc., the "concentrate" extract containing 15 U.S.P. units in 1 cc. of material. These extracts are said not to contain iron.

U S P units) of liver was given at intervals of from 7 to 12 days until the red count reached 5 million. Liver extract was given by intramuscular injection, and in no case was iron given, nor was liver given by mouth.

Plasma and total blood volumes and hematocrits were determined by methods previously described (10), venous pressures by a direct manometric method (11), circulation time by means of decholin (12), and hemoglobin content of venous blood by the method of Osgood and Haskins (13). Surface area was taken as the basis for the prediction of normal blood volume. Normal values for hematocrit were taken as 44 per cent and 40 per cent (14), for red blood cell counts as 5,480,000 and 4,920,000 cells per c mm of blood, and for hemoglobin content of venous blood as 16.03 and 14.21 grams per 100 cc of whole blood (15), for men and women respectively. Based on the above, normal values were computed for mean corpuscular volume as 80.3 and 81.3 cubic microns, for mean corpuscular hemoglobin as 29.3 and 28.9 micrograms, and for mean corpuscular hemoglobin concentration as 36.5 and 35.5 grams per 100 cc. of packed red cells for men and women respectively. Total circulating hemoglobin was computed by multiplying the hemoglobin content in grams per 100 cc. of blood by the total blood volume in hundreds of cubic centimeters.

RESULTS

All of these patients made complete recoveries, the red blood cell count rising to about 5 million in from 40 to 60 days after the beginning of treatment. During the first 28 days of treatment the rate of recovery of individual patients varied somewhat as shown in Figure 1. During the first 10 days the response of 2 of the 3 patients (Cases 165, 216, and 283), receiving large initial doses, was more rapid than in those receiving small amounts of "concentrate" extract daily. Case 218, receiving an initial single dose of material derived from 50 grams of liver (7.5 U S P units), had a response comparable to those patients receiving divided doses of "concentrate" extract. The degree of recovery at 28 days, in terms of percentage of deficit from normal in red cell count existing before therapy, varied somewhat with the

total dosage during this period but in general was proportionate to the amount of liver given.

Blood volume

The data obtained are presented in Table I. In Figure 2 are shown the absolute plasma, circulating red blood cell, and total blood volume at red blood cell count levels of from 1 to 5 million. Almost without exception at the initial determination, plasma volume was higher, and circulating red cell and total volume were lower than the average normal values (14) for men and women. In every case plasma volume fell and red cell and total blood volume rose as the red blood cell count increased to 5 million under liver therapy.

When recovery was complete, average plasma volume had decreased from initial levels by 473 cc and 460 cc, average circulating red cell volume had increased by 1570 cc and 1065 cc, and average total blood volume had increased by 928 cc. and 508 cc, in the males and females respectively. In no individual case was any marked deviation from the general trend observed. The increase in circulating red cell volume amounted to about 200 per cent and 150 per cent above the amount present before treatment was started in the males and females respectively.

In relation to normal blood volume predicted on the basis of surface area there was, at various levels of anemia, some individual variation in the percentage of deviation from normal in plasma, circulating red cell and total blood volume, the extremes of this variation being within about plus or minus 10 per cent of the average values for the group, which are shown in Figure 3. At a red blood cell count level of 1.5 million the plasma volume was 30 per cent above, the red cell volume 68 per cent below, and the total blood volume 16 per cent below normal. During recovery on liver therapy the fall in plasma volume and the rise in red cell and total blood volume were practically in linear relationship to the increase in the red blood cell count, and at the time the red blood cell count had reached 5 million, the plasma, circulating red cell, and total blood volume all were within normal limits. A similar relationship in the changes in plasma, circulating red cell, and total blood volume to the increase in the hematocrit level was observed.

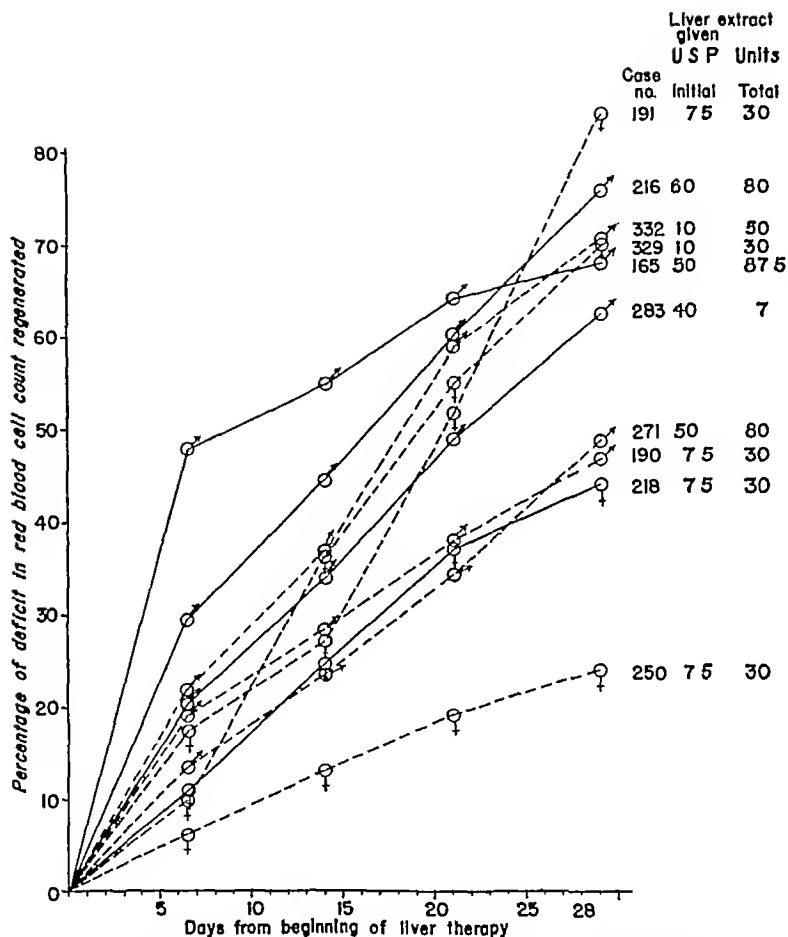


FIG. 1 THE COURSE OF THE RED BLOOD CELL COUNT EXPRESSED IN TERMS OF PERCENTAGE OF INITIAL DEFICIT REGENERATED IN 28 DAYS IN 10 CASES OF PERNICIOUS ANEMIA TREATED BY INTRAMUSCULAR INJECTIONS OF LIVER EXTRACT

The amount of liver in U S P units given as an initial dose and the total given during the period shown are listed opposite case numbers at the right. The solid lines represent the cases given large single initial doses and the broken lines the cases given 10 daily small doses of a concentrate extract.

TABLE I

Plasma, circulating red cell and total blood volume, red cell count and hemoglobin, venous pressure and circulation time in 10 cases of pernicious anemia

Case number	Date	Age	Sex	Height	Weight	Surface area	Venous pressure	Circulation time	Red blood cells	Hemoglobin	Hematocrit	Normal blood volume based on surface area			Determined blood volume			Percentage deviation from predicted normal volume						Normal total hemoglobin	Total hemoglobin	Percentage deviation from normal total hemoglobin			
												Plasma	Red cells	Total blood	Plasma	Red cells	Total blood	Plasma		Red cells		Total blood							
																		cc	cc	cc	cc	cc	cc			per cent	per cent	per cent	per cent
165A	April 7 1936	42	M	176.5	56.7	1.69	75	14	1.67	4.47	2705	2205	4925	4050	650	4700	49.7	70.6	18.1	77.1	790	210	77.1	790	210	77.1	77.1	77.1	
165B	April 16 1936			56.8	67.0	1.81	45	22	2.60	7.10	28.6	2930	5300	3300	2360	5660	12.6	35.3	0.80	55.5	850	704	55.5	850	704	55.5	55.5	5.4	
165C	May 25 1936								5.16	12.42	41.6	2930	5300	3300	2360	5660	12.6	0.4	6.37	5.4	850	704	5.4	850	704	5.4	5.4	5.4	
190A	June 5 1936	59	M	162.0	53.3	1.55	30	34	2.26	6.90	26.7	2340	4310	3280	1190	4470	40.2	39.5	3.71	55.4	691	308	55.4	691	308	55.4	55.4	55.4	
190B	July 14 1936			49.2	52.3	1.54	40	44	3.74	9.80	36.6	2240	4810	4050	2870	1650	4530	28.1	8.3	11.80	31.6	650	445	31.6	650	445	31.6	31.6	
190C	December 28 1936								5.33	14.63	41.5	2350	4300	2860	2020	4880	21.7	3.06	13.50	3.6	690	715	3.6	690	715	3.6	3.6	3.6	
191A	June 3 1936	66	F	147.5	64.0	1.56	110	15	1.22	5.52	15.3	2400	4000	3500	630	4130	45.7	60.6	3.25	60.2	570	227	60.2	570	227	60.2	60.2	60.2	
191B	July 7 1936			61.8	61.8	1.53	90	23	3.38	10.25	33.4	2370	4000	3500	630	4170	17.3	12.0	5.57	24.2	563	427	24.2	563	427	24.2	24.2	24.2	
191C	January 13 1937			66.9	66.9	1.59	70	20	5.50	13.05	39.8	2470	4300	3100	2575	1705	4280	4.2	4.5	4.38	4.1	584	560	4.1	584	560	4.1	4.1	4.1
216A	June 27 1936	64	M	158.5	55.8	1.58	50	13	1.04	4.83	14.9	2460	4450	3760	690	4900	3250	75.4	9.0		77.9	714	157	77.9	714	157	77.9	77.9	77.9
216B	January 26 1936			62.2	62.2	1.62	60	35	5.17	15.09	44.1	2455	4625	2505	1975	4480	2.2		3.1	8.9	742	676	8.9	742	676	8.9	8.9	8.9	
218A	July 1 1936	49	F	166.8	70.8	1.78	65	23	1.76	5.60	16.2	2485	4150	2740	530	3290	10.3	68.1	21.2	68.2	592	183	68.2	592	183	68.2	68.2	68.2	
218B	January 20 1937								5.10	15.95	40.8	2505	4180	2740	530	3290	10.3	0.9	4.18	8.7	596	648	8.7	596	648	8.7	8.7	8.7	
250A	November 28 1936	74	F	156.1	64.0	1.62	55	17	1.56	7.59	17.2	2475	4050	3125	2590	640	3130	4.7	61.3	24.1	59.4	587	238	59.4	587	238	59.4	59.4	59.4
250B	December 19 1936			63.0	63.0	1.61	20	16	2.59	9.38	26.3	2460	4100	2400	860	3260		47.5	20.5	47.6	584	306	47.6	584	306	47.6	47.6	47.6	
250C	January 9 1937			64.5	64.5	1.63	60	21	3.45	12.02	34.9	2475	4125	2540	1360	3900	2.6	17.6	5.5	58.7	587	470	58.7	587	470	58.7	58.7	58.7	
250D	February 23 1937			67.3	67.3	1.66	35	25	5.14	16.18	40.7	2490	4160	2360	1610	3970		3.0	5.22	8.1	592	640	8.1	592	640	8.1	8.1	8.1	
271A	January 7 1937	72	M	177.5	67.8	1.82	45	19	2.17	9.50	20.5	2985	3415	3340	860	4200	12.2	64.4	22.2	53.9	866	399	53.9	866	399	53.9	53.9	53.9	
271B	January 14 1937			65.8	65.8	1.80	45	20	2.84	11.40	24.8	2905	3350	3350	860	4200	12.2	51.7	11.3	31.6	854	538	31.6	854	538	31.6	31.6	31.6	
271C	February 10 1937			72.3	72.3	1.88	85	19	3.80	11.25	35.9	3115	3510	3150	1760	4910	1.1	29.8	12.7	6.1	905	552	6.1	905	552	6.1	6.1	6.1	
283A	February 8 1937			63.2	63.2	1.66	30	16	1.05	3.73	11.4	2645	4775	3590	480	4120	37.8	77.5	13.7	79.9	765	154	79.9	765	154	79.9	79.9	79.9	
283B	February 15 1937	70	M	161.5	61.2	1.63	40	14	1.97	5.58	19.4	2585	4090	3675	860	4450	38.9	58.9	4.8	67.0	750	248	67.0	750	248	67.0	67.0	67.0	
283C	March 6 1937			67.2	67.2	1.70	40	25	3.07	8.56	29.2	2740	4950	3690	1530	5220	34.6	30.7	5.5	43.7	794	447	43.7	794	447	43.7	43.7	43.7	
283D	April 28 1937			67.2	67.2	1.70	30	25	4.69	15.00	39.1	2740	4950	3690	1530	5400	20.2	4.5	11.10	2.1	794	811	2.1	794	811	2.1	2.1	2.1	
329A	November 30 1937	63	F	150.0	60.2	1.54	80	12	0.83	4.28	13.8	2385	3975	3190	510	3700	33.7	67.9	6.8	71.8	565	159	71.8	565	159	71.8	71.8	71.8	
329B	December 9 1937			60.6	60.6	1.54	50	13	2.25	7.39	22.9	2385	3975	3190	510	3700	33.7	44.6	3.7	49.9	565	283	49.9	565	283	49.9	49.9	49.9	
329C	December 22 1937			61.0	61.0	1.54	40	16	3.32	11.55	32.4	2385	3975	3190	510	3700	33.7	16.7	3.15	16.8	565	470	16.8	565	470	16.8	16.8	16.8	
332A	January 6 1938	61	M	172.5	56.3	1.66	95	18	1.38	5.26	17.9	2660	4800	3670	800	4470	38.0	62.7	6.5	69.5	770	235	69.5	770	235	69.5	69.5	69.5	
332B	January 26 1938			56.2	56.2	1.62	30	24	3.07	12.10	35.0	2660	4800	3670	800	4470	38.0	20.5	0.8	23.9	770	586	23.9	770	586	23.9	23.9	23.9	
332C	March 10 1938			56.4	56.4	1.62		17	5.08	16.05	40.8	2660	4800	3670	800	4470	38.0	6.5	2.45	2.3	770	788	2.3	770	788	2.3	2.3	2.3	

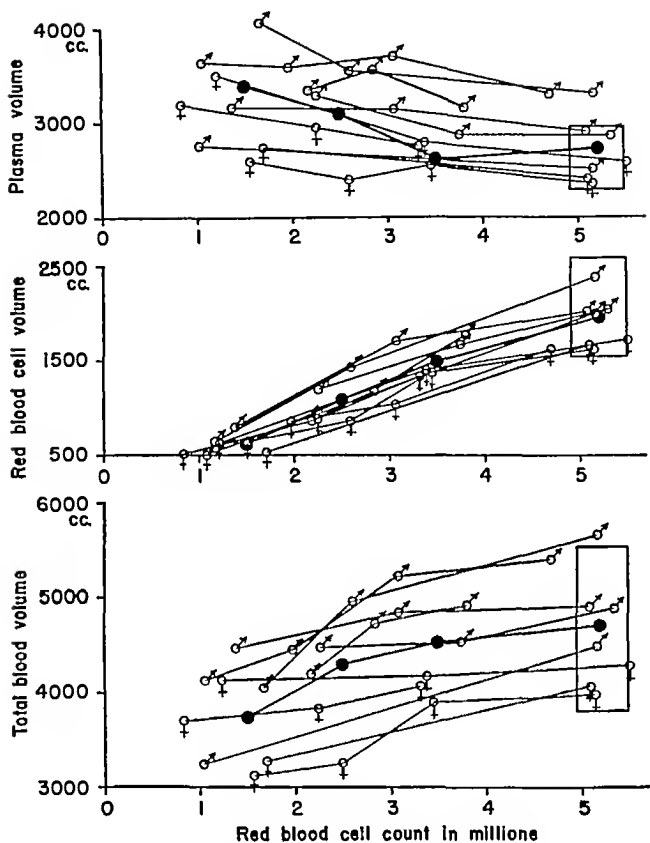


FIG. 2 ABSOLUTE PLASMA, CIRCULATING RED CELL, AND TOTAL BLOOD VOLUME IN 10 CASES OF PERNICIOUS ANEMIA DURING RECOVERY ON INTRAMUSCULAR LIVER EXTRACT THERAPY

The initial determinations were made just prior to the beginning of treatment. Normal limits are indicated by the rectangles at the right of the figure, the upper and lower borders representing the average normal volume levels and the right and left borders representing the average normal red cell counts for males and females respectively. The heavy lines represent averages for the group. As the red cell count rises plasma volume diminishes and circulating red cell and total blood volume increase, all returning to normal when the erythrocyte count reaches 5 million.

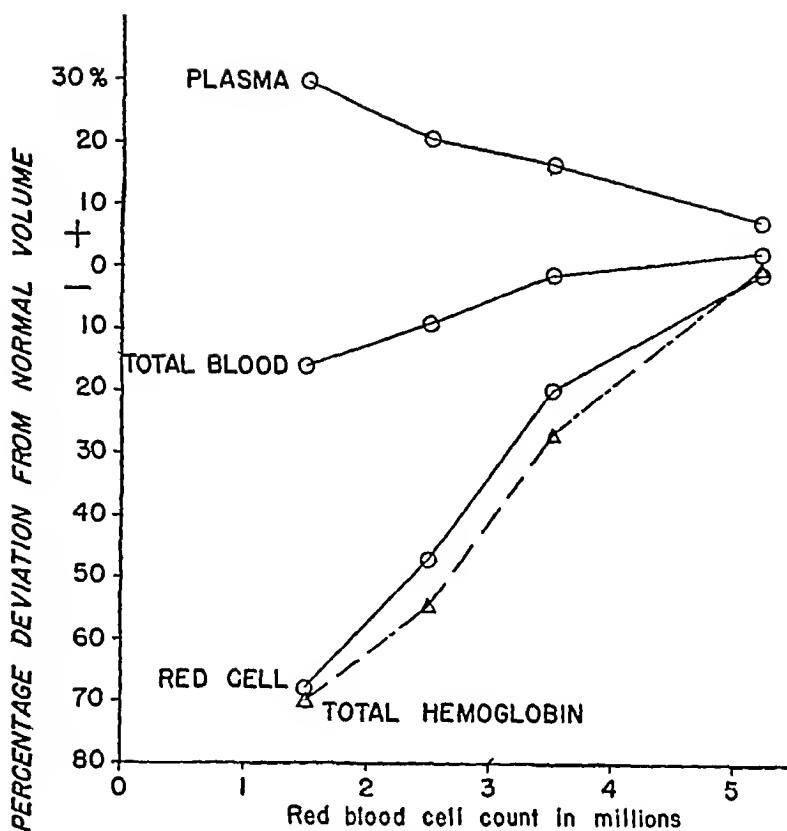


FIG 3 THE PERCENTAGE DEVIATION FROM PREDICTED NORMAL VALUES IN PLASMA, CIRCULATING RED CELL AND TOTAL BLOOD VOLUME, AND TOTAL CIRCULATING HEMOGLOBIN IN SEVERE PERNICIOUS ANEMIA AND DURING RECOVERY ON LIVER THERAPY

The regeneration of hemoglobin is slower than the regeneration of red cells

Hemoglobin

Total circulating hemoglobin was extremely low during the severe stages of anemia, having been, on an average, 238 and 202 grams, in contrast to average normal amounts of 877 and 540 grams, in the males and females respectively. After recovery, total circulating hemoglobin had increased over initial levels on an average of 519 grams, or about 220 per cent in the males and 411 grams or about 200 per cent in the females. In terms of percentage deviation from normal the course of the total circulating hemoglobin bore a linear relationship to the increase in red cell count as shown in Figure 3, but while the curve of increase in total circulating hemoglobin paralleled the corresponding curve of circulating red cell volume, it definitely lagged behind during the early and middle stages of therapy.

In Table II are shown changes in mean corpuscular volume and hemoglobin, mean corpuscular hemoglobin concentration, and in the volume, color and saturation indices. The averages of volume and hemoglobin corpuscular measurements with reference to the course of the total circulating red cell volume as the red blood cell count rose to 5 million cells are shown in Figure 4.

Venous pressure and circulation time

Venous pressures varied considerably during the course of treatment, having a tendency to be in the high range of normal during the anemic stages and to fall slightly with clinical improvement as shown in Figure 5. However, there were considerable individual variations, ranging from 30 to 110 mm of water, in initial determina-

TABLE II

Individual cell volume and hemoglobin measurements and volume color and saturation indices in 10 cases of pernicious anemia

Case number	Red blood cells	Hemoglobin	Hematocrit	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular hemoglobin concentration	Volume index	Color index	Saturation index
	millions per c. mm.	grams per 100 cc.	per cent of cells	cubic microns	micrograms per 100 cc.	grams per 100 cc.			
185A	1.67	4.47	12.8	82.7	26.8	32.3	1.033	0.821	0.260
185B	2.80	7.10	23.0	110.0	37.3	34.5	1.403	0.832	0.663
165D	8.15	13.42	41.6	80.6	29.1	39.9	1.003	0.822	0.819
190A	2.26	6.80	20.7	117.8	30.5	25.6	1.478	1.043	0.707
190B	2.74	9.10	26.0	97.5	35.3	36.2	1.229	0.977	0.735
190C	6.23	14.63	41.6	77.5	27.4	35.3	0.970	0.940	0.970
191A	1.22	4.82	15.3	128.0	45.0	48.3	1.605	1.610	1.005
191B	2.83	10.25	32.4	95.8	30.3	30.3	1.215	1.050	0.853
191C	3.50	10.05	39.8	79.3	28.6	28.7	0.867	0.880	0.823
216A	1.04	4.83	18.1	146.0	46.5	23.0	1.806	1.858	0.578
216B	6.17	15.09	44.1	83.3	29.3	34.3	1.090	0.960	0.943
218A	1.70	5.80	16.3	103.3	32.9	34.7	1.170	1.133	0.970
218B	5.10	13.86	40.8	80.1	32.7	39.0	0.937	1.022	1.085
230A	1.46	7.99	17.3	110.2	48.8	44.1	1.360	1.635	1.240
230B	2.84	9.25	29.3	100.3	38.3	37.1	1.260	1.250	0.953
230C	3.45	12.02	34.0	100.1	34.8	34.7	1.245	1.206	0.947
230D	5.14	13.18	40.7	79.3	31.4	39.5	0.972	1.000	1.120
271A	2.17	9.80	20.5	94.4	43.2	45.2	1.170	1.424	1.272
271B	2.84	11.40	27.3	87.3	40.2	46.0	1.083	1.272	1.203
271C	5.03	11.25	35.9	77.3	29.5	31.8	1.178	1.030	0.977
282A	1.50	3.73	11.4	108.6	35.7	32.7	1.343	1.318	0.905
282B	1.97	4.88	10.4	94.3	25.3	28.6	1.230	0.972	0.790
282C	2.07	8.54	29.3	93.3	37.9	39.0	1.195	0.963	0.903
282D	4.67	15.00	39.1	69.3	31.1	38.3	1.033	1.106	1.068
286A	0.82	4.28	18.8	166.3	51.8	31.0	2.050	1.763	0.871
270B	2.75	7.39	22.9	101.6	33.8	32.3	1.234	1.138	0.907
282C	2.22	11.85	32.4	87.6	35.5	39.0	1.200	1.268	1.006
272A	1.29	5.30	17.3	130.0	38.1	29.4	1.618	1.900	0.905
272B	3.07	12.10	25.0	114.0	39.4	34.6	1.420	1.350	0.950
272C	5.08	16.05	40.8	80.3	31.6	39.3	1.000	1.077	1.077

tions of venous pressure, and in the direction and degree of changes observed during therapy

Circulation time was definitely decreased in the anemic stages of the disease as shown in Figure 5, and on the average, increased during therapy in a linear relationship to the increase in the red cell count, returning to normal when recovery was complete.

DISCUSSION

In contrast to the conflicting observations reported in the literature the findings herein presented exhibit a striking consistency in the degree of abnormality of plasma, red blood cell and total blood volume during the severe stages of pernicious anemia and in the behavior of the component elements of the blood volume both as to absolute values and relative changes during recovery on liver extract therapy. Pernicious anemia is char-

acterized by a hydremic hypovolemia, and the degree of hydremia and hypovolemia and of reduction from normal in the circulating red cell volume and total hemoglobin is in direct relationship to the severity of the disease as evidenced by the levels of the red cell count or hematocrit, and hemoglobin

A somewhat different situation prevails in the hypochromic anemias. In a group of 31 patients with secondary anemias, 9 of whom had an anemia due to chronic blood loss, 1 due to acute nephritis, 3 due to chronic vascular nephritis, 9 due to chronic glomerular nephritis and 9 due to so-called "nephrosis," the author has found no definite relationship between the degree of deviation from normal total blood volume and the red blood cell count level, about one third of the group having total blood volumes from 0 to 15 per cent above and the remainder having total blood volumes from 0 to 18 per cent below normal the average value for the entire group being only slightly below normal. However all of these patients had subnormal circulating red blood cell volumes, and, in resemblance to the group of patients with pernicious anemia herein studied the relationship of the percentage deviation from normal circulating red cell volume to the red blood cell count was such that the slope of the curve of this deviation was the same in both groups. It would seem that the patient with pernicious anemia, in contrast to the patient with a secondary anemia, has definite limits to the extent to which total blood volume, diminished by a reduction in the circulating red cell volume, can be augmented by blood hydration. Other than being possibly due to chronic dehydration, the cause of this difference is not clear and merits further investigation.

The marked reduction in total circulating hemoglobin observed by other workers is confirmed by this study. The patients in the group studied when they first came under medical observation, had on an average only one-third of their normal total circulating hemoglobin, and yet none of them presented symptoms of marked respiratory distress. A discussion of the "compensatory" mechanism by which patients with pernicious anemia carry on fairly adequate respiratory function is beyond the scope of this paper. That the efficiency of the lowered oxygen capacity of the blood is increased is a widely accepted belief. The

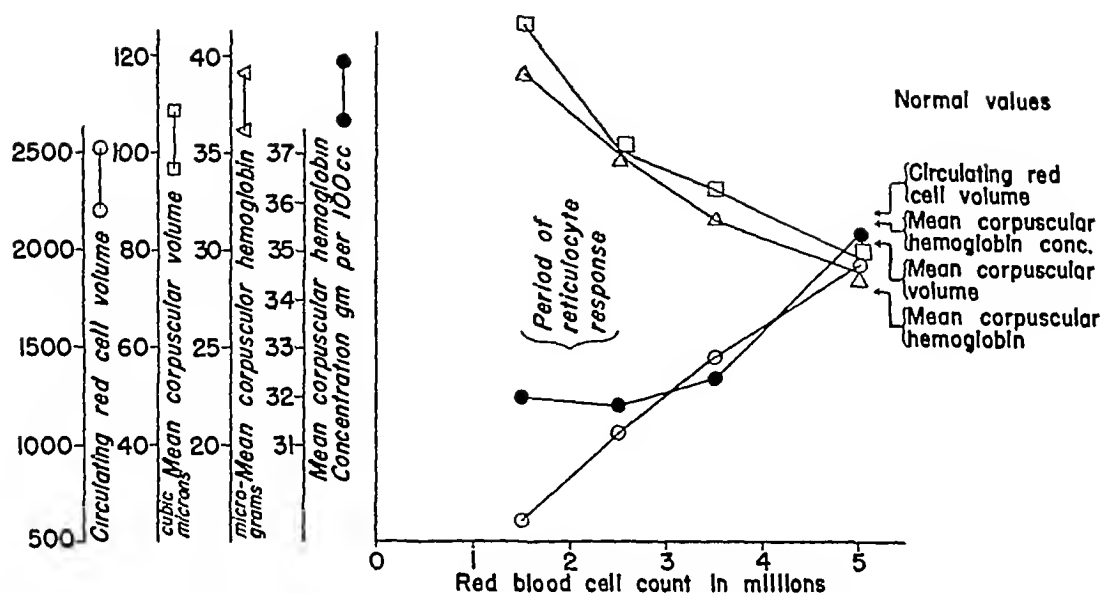


FIG. 4 CHANGES IN THE SIZE AND HEMOGLOBIN CONTENT AND CONCENTRATION OF THE INDIVIDUAL RED CELL, AND IN THE SATURATION INDEX DURING RECOVERY FROM PERNICIOUS ANEMIA ON LIVER THERAPY, SHOWN IN RELATION TO THE INCREASE IN THE CIRCULATING RED CELL VOLUME

The lines represent average values for the group of 10 cases. While the individual cell is larger and contains more hemoglobin than a normal red cell, the total red cell mass, in comparison to normal, is definitely deficient in hemoglobin, and this deficiency is not fully met until recovery is almost complete.

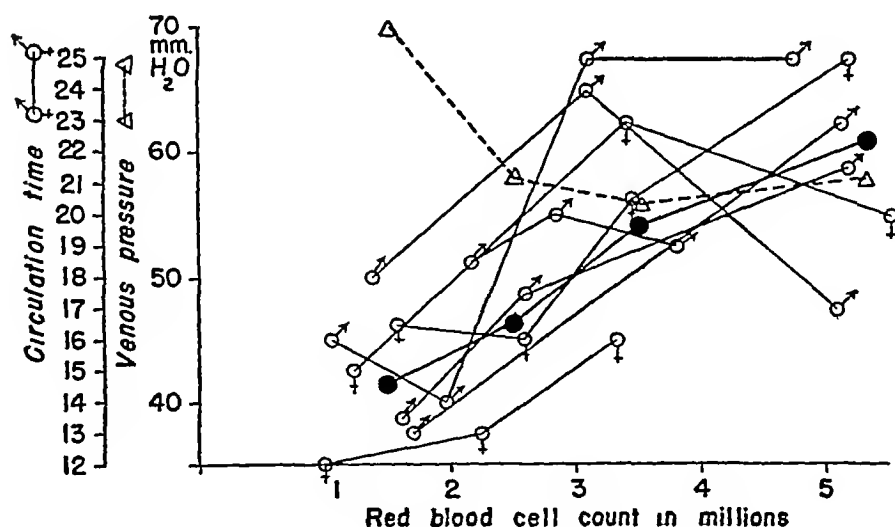


FIG. 5 INCREASE IN THE CIRCULATION TIME AS THE RED CELL COUNT RISES

The line connecting the black circles is the average trend of circulation time, that connecting the triangles the average trend of venous pressure. The changes in venous pressure are of no clinical significance.

finding of an increased speed of blood flow in this group of patients (see Figure 5) is in keeping with the observation of Blumgart *et al* (16). Better oxygen utilization as evidenced by an increase in the arteriovenous difference in oxygen content of the blood, has been demonstrated by Henderson (17).

In this series of cases the size, hemoglobin content, and concentration of the individual red cell and the volume, color, and saturation indices are in keeping with those reported in the literature. Thus Wintrobe (18) found an average mean corpuscular volume of 116 cubic microns in patients with pernicious anemia with erythrocyte counts ranging from 0.59 to 4.03 million, in comparison to a normal range of from 70 to 98 cubic microns (19), and Haden (20) found the mean corpuscular volume in pernicious anemia to range from 108 to 163 cubic microns. These same authors found the mean corpuscular hemoglobin to average 39 and 40.1 micrograms both values being well above normal. The volume index has been found to be greater than 1 by Capps (21) and Haden (22) and color index greater than 1 by Goldhamer, *et al* (23). Yet Haden (24) reported an average saturation index of 0.92 in 50 cases, indicating that the red cells did not contain their maximum capacity of hemoglobin and certainly were not supersaturated as the color index would suggest.

The data illustrated in Figure 4 are of interest in this connection. Both mean corpuscular volume and hemoglobin are definitely above normal in the severe stages of anemia and decrease approaching normal as the red cell count rises. Mean corpuscular hemoglobin concentration is an expression of the hemoglobin content of a unit mass, not of whole blood but of red cells, and this value is decidedly below normal in the severe stages, approaching normal only after the red cell count has reached about 3 million.

It is, therefore, apparent that in pernicious anemia while the individual cell is larger and contains more hemoglobin than a normal red cell the total red cell mass in comparison to an equal mass of normal red cells is definitely deficient in hemoglobin and that on liver therapy alone, this deficiency is not met until recovery is almost complete. It is of interest that this failure of the total red cell mass to attain its normal complement of hemoglobin occurs at a time, following effective

liver therapy, when red cells are being rapidly regenerated. It cannot be entirely accounted for by decreases in the mean corpuscular volume and hemoglobin, since, as shown in Figure 3 in terms of percentage of normal, total hemoglobin is less than total circulating red cell volume until the erythrocyte count reaches normal.

Two explanations of this difference in rate of regeneration of red cells and hemoglobin suggest themselves. The formation of hemoglobin may be slower than the development of new red cells and hence inevitably proceed at a slower rate. The bone marrow of pernicious anemia is hyperplastic, and it is characteristic that the erythrocyte is arrested in its development in this disease. The effective principle of liver is thought to check this developmental inhibition so that a large quantity of dormant cells rapidly mature and are liberated early in the course of treatment.

Another possibility is that a true iron deficiency may exist in the severe stages of the disease, so that a real shortage of hemoglobin building substances may delay the synthesis of hemoglobin. Murphy, Lynch, and Howard (25) concluded that during a relapse the "iron index" (whole blood iron divided by the red cell count in millions) was above normal, approaching normal during recovery. Reich and Tiedemann (26) also found a normal iron volume index in pernicious anemia but regarded this as evidence that iron therapy was of no value in this disease since "the red cells are already saturated with iron." Moore and Doan (27), however, clearly showed that the plasma iron which they considered to be iron in the form most available for hemoglobin synthesis, while normal or above during a relapse, fell rapidly to subnormal levels on the institution of liver therapy and remained low throughout the period of reticulocyte response and until the erythrocyte count had reached between 3 and 4 million cells after which it gradually rose. Thus, while the red cells present in the blood during a relapse probably have a normal complement of iron the lowering of the plasma iron accompanying the tremendous new production of hemoglobin during treatment is direct evidence of a depletion of stored iron in the severe stages of the disease. It is possible that the patient with pernicious anemia should receive therapy directed to, in addition to, ing an iron deficit in addition to

the effective substance contained in liver Murphy (28) has shown that the response of a group of patients with pernicious anemia treated with iron supplementing liver extract was definitely better than that of a control group receiving liver therapy alone

The immediate increase in reticulocytes characteristic of liver therapy in pernicious anemia has led to the practice of using the reticulocyte response as a basis for judging the potency of any liver preparation That the height of the rise of the reticulocytes varies with the initial level of red blood cells when liver is given by mouth was pointed out by Minot, Cohn, Murphy, and Lawson (29)

Bethell and Goldhamer (30) described a similar characteristic of the reticulocyte response in 39 patients receiving a single dose of liver extract (an amount derived from 100 grams of liver) intravenously In a larger series of cases given liver extract intramuscularly at intervals of from 1 to 7 days, the totaling amount averaging about 1 USP unit a day, Isaacs and Friedman (31) confirmed these findings Based on this characteristic the Council on Pharmacy and Chemistry of the American Medical Association has adopted a standard test curve (32) for evaluating the potency of liver extracts to be used in the treatment of pernicious anemia

In Table III is shown the initial reticulocyte and

TABLE III
Increases in the reticulocyte and red blood cell counts in 10 patients following intramuscular liver extract therapy

Case number	Total amount of liver extract	Ten daily equal doses	Single dose		Before treatment	Days after beginning therapy									
						1	2	3	4	5	6	7	8	9	10
165	U.S.P. units 50.0		x	R*		20	80	150	291	497	407	283	140	26	
				C†	17						24				
190	7.5	x		R	10		20	20	16	12	28	92	106	44	32
				C	23				25					30	
191	7.5	x		R		56	48	58	54	60	104	155	140	156	104
				C	12							18			19
216	60.0		x	R	18		26	16	160	170	245	241	122	112	96
				C	10				11			24			24
218	7.5		x	R	28	28	62	64	138	182	210	146			
				C	13				16			17			
250	7.5	x		R	12	12	16	22	44		86	120	160	186	94
				C	16				17				18	18	
271	7.5	x		R	20	33	28		40	60	84	106	178	102	87
				C	16				19			22		20	25
283	40.0		x	R	04	04	07	126	304	362	268	330			
				C	11							20			
329	10.0	x		R	18	21	31	45	61	131	230	330	269	219	220
				C	10								15		
332	10.0	x		R	03	04	06	16	20	45	79	127	280	270	296
				C	14								23		

* Reticulocyte count in per cent of red blood cell count

† Red blood cell count in millions per c. mm

red blood cell counts and the course of these determinations during the first 10 days of treatment in the patients in this series with reference to the number of U S P units administered and the size and frequency of dosage given. In Figure 6 the reticulocyte level at the time of the maximum response is shown in comparison to the expected maximum based on the standard test curve of the American Medical Association and in addition to the absolute increase in red cell count, and the percentage of the deficit in red blood cell count, total circulating hemoglobin, and circulating red blood cell volume present at the beginning of therapy regenerated in 10 days.

In the 6 cases given small daily doses the reticulocyte response was satisfactory in all but Cases 191 and 329 and yet the production of new cells and hemoglobin after 10 days of treatment in these cases was above the average of the entire group, and on continued treatment for 28 days with the same extract initially used (see Figure 1) the red cell count was definitely above the average for the entire group.

On the other hand in Cases 250 and 271 new production of red cells and hemoglobin was definitely below average both at the 10 and 28-day periods in spite of a reticulocyte response that was well up to standard test requirements.

Thus in 4 of the 6 cases receiving 10 daily doses the conformance of the reticulocyte count to the standard test curve bore no constant relationship to the regeneration of blood either during the reticulocyte rise or during a subsequent period of about 3 weeks thereafter.

A comparison of the 4 cases given large single doses as initial treatment is of interest. In all these cases the reticulocyte response was prompt and while it cannot be directly referred to the standard test curve (initial red cell counts being considered) was on the whole higher than that of the group receiving 10 daily divided doses (see Table III). Yet the production of red cells and hemoglobin in terms of deficit regenerated was below average at 10 days in Cases 216 and 283, and about average in Cases 165 and 218. Eighteen days later, during which interval further large doses were given to 3 cases (Cases 265, 216 and 283), the increase in red cells was only a little better than the average of the group receiving divided doses.

It would appear from the data presented that the reticulocyte response experienced by these patients did not bear a constant relationship to the degree of improvement in their blood as a whole either during the phase of reticulocyte activity or subsequent thereto. It should also be stated that the reticulocyte response is transient and cannot serve as an index of the therapeutic effectiveness of a liver preparation throughout the entire course of recovery or during maintenance therapy.

The striking linear relationship of the red blood cell count and hematocrit to the percentage of normal circulating red blood cell volume and total hemoglobin respectively observed in this study indicates that, for purposes of following the clinical course of the patient, the red cell count or hematocrit are good indicators of the degree of return to normal levels of the circulating red cell volume, and the hemoglobin determination is a good indicator of the degree of the return to normal of the total hemoglobin content of the blood. It is suggested that a useful interpretation of increases in red cell count and hemoglobin is in terms of the percentage of the deficit existing before the beginning of treatment regenerated.

These findings perhaps suggest the wisdom of evaluating the potency of a liver preparation upon the course of red cell count and hemoglobin during the entire period of recovery, in addition to the conformance of the transient reticulocyte rise to a standard test curve.

CONCLUSIONS

1 Pernicious anemia is characterized by a hydremic hypovolemia in the severe stages. In the group of 10 cases studied the average plasma volume was 30 per cent above, circulating red blood cell volume 68 per cent below, total blood volume 14 per cent below and total hemoglobin 70 per cent below normal at a red blood cell level of 1.5 million.

2 Under treatment with a potent liver extract, plasma circulating red blood cell, total blood volume, and total circulating hemoglobin returned to within normal limits when the red cell count reached normal, and these blood components changed in a linear relationship to the change in the red cell count and hematocrit.

3 In the severe stages of anemia, although the individual red cells were larger than

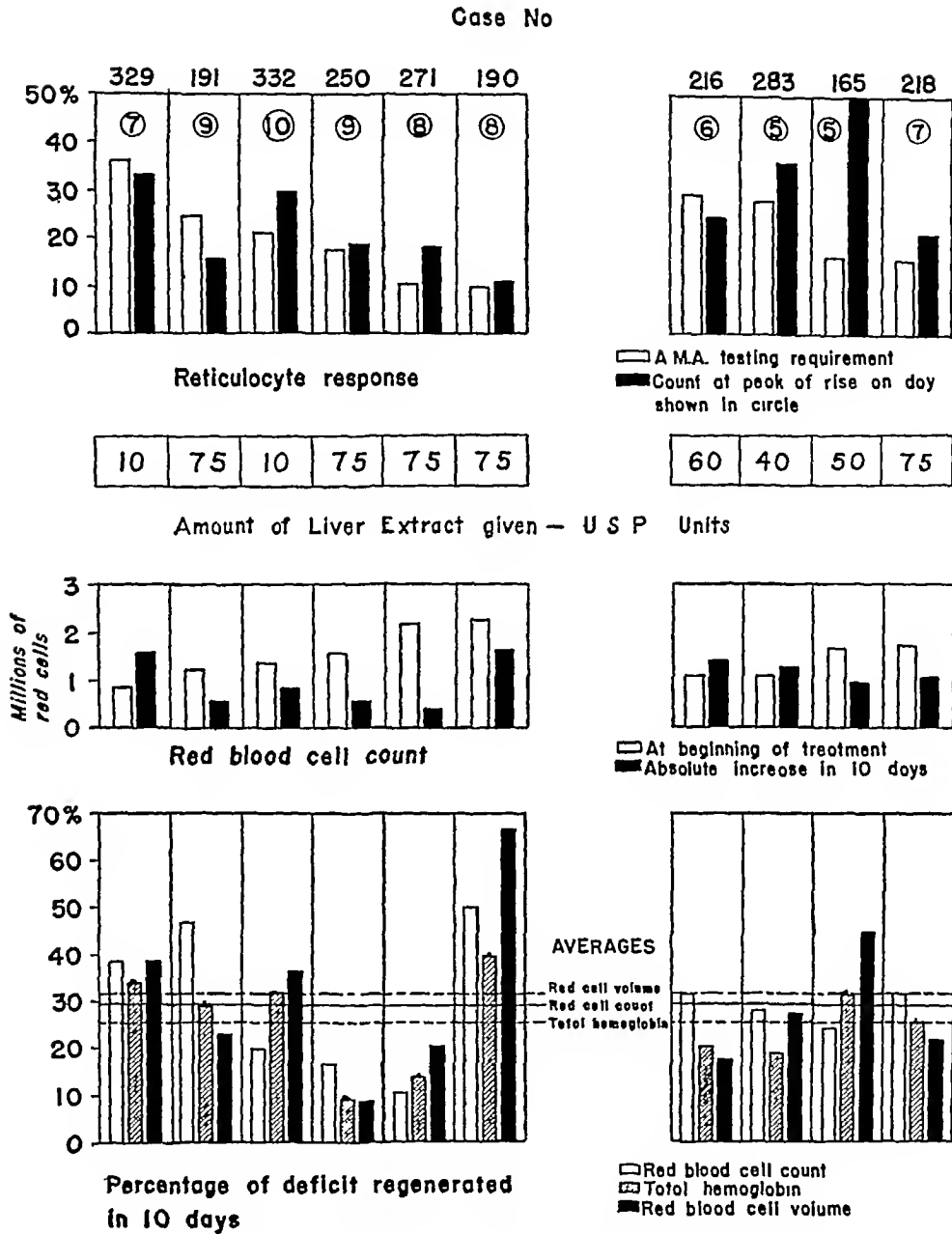


FIG 6 REGENERATION OF BLOOD FOLLOWING INTRAMUSCULAR LIVER THERAPY

Cases given 10 daily doses of concentrate are grouped at the left of the chart, those given single large doses at the right. The relationship of the conformance of the reticulocyte maximum to the regeneration of the blood as a whole in terms of the percentage of the deficit in red cells and hemoglobin present at the beginning of therapy regenerated in 10 days is discussed in the text.

contained more hemoglobin than a normal cell, the hemoglobin content of a unit mass of red cells was less than that of an equal unit mass of normal red cells. There is in pernicious anemia, a relative as well as absolute deficiency in hemoglobin.

4 In the cases studied, the magnitude of the reticulocyte response following treatment with a potent extract bore no constant relation to the concomitant or subsequent improvement in the blood in terms of the percentage of regeneration of the deficit in circulating red cell volume or total hemoglobin. It is suggested that the present practice of evaluating the potency of any liver preparation on the basis of the conformance of the reticulocyte response to a standard test curve might well be supplemented by consideration of the course of the red blood cell count and hemoglobin over the entire period of recovery.

The author wishes to thank Dr William P. Murphy for his interest and encouragement in this study and Miss Isobel Howard for her painstaking technical assistance.

BIBLIOGRAPHY

1. Bock, A. V. The constancy of the volume of the blood plasma. *Arch. Int. Med.*, 1921 27 83
2. Denny, G. P. The blood volume in pernicious anemia. *Arch. Int. Med.*, 1921 27 38.
3. Murphy W. P., Monroe R., and Fitz, R., Changes in the composition of the blood in pernicious anemia. *J. A. M. A.* 1927 88 1209
4. Keith, N. M., The total circulating blood and plasma in cases of chronic anemia. *Am. J. M. Sc.*, 1923 165 174
5. Rusynak, S. Untersuchungen zur Frage der gesamt Blutmenge des Menschen unter normalen und pathologische Verhältnissen. *Deutsches Arch. f. klin. Med.* 1927 67 186.
6. De Weaselow O. L. V., and Bamforth J. The blood and plasma volumes in pernicious anemia. *Lancet*, 1928 1 1016
7. Smith J. L. The blood in disease. *Tr. Path. Soc. London*, 1900 51 311
8. Hölbbol S. A., Untersuchungen über den Einfluss der Lebertherapie auf die grosse der Blutmenge bei Patienten mit Anämia Perniciosa. *Acta med. Scandinav.* 1929 Supp. 34 90
9. Gregersen M. I., Gibson J. G., and Stead, E. A., Plasma volume determination with dyes errors in colorimetry use of the blue dye T-1824. *Am. J. Physiol.*, 1935 113 54
10. Gibson J. G. 2d, and Evans, W. A., Jr. Clinical studies of the blood volume. I. Clinical application of a method employing the blue azo dye "Evans Blue" and the spectrophotometer. *J. Clin. Invest.*, 1937 16, 301
11. Evans Wm. Venous pressure. *New England J. Med.*, 1932, 207, 1934
12. Wintemitz, M., Deutsch, J., and Brüll, Z., Eine klinisch brauchbare Bestimmungsmethode der Blutumlaufzeit mittels Decholinjektion. *Med. Klin.*, 1931 27 986.
13. Osgood E. E., and Haskins, H. B., A new permanent standard for estimation of hemoglobin by the acid hematin method. *J. Biol. Chem.*, 1923 57 107
14. Gibson, J. G., 2d, and Evans, W. A., Jr., Clinical studies of the blood volume. II The relation of plasma and total blood volume to venous pressure, blood velocity rate, physical measurements age and sex in ninety normal humans. *J. Clin. Invest.*, 1937 16, 317
15. Murphy W. P., and Howard, I. M., The iron content of crystals of hemoglobin prepared from human blood. (In preparation.)
16. Blumgart, H. L., Gargill S. L., and Gilligan, D. R., Studies on the velocity of blood flow. XV The velocity of blood flow and other aspects of the circulation in patients with "primary" and secondary anemia and in two patients with polycythemia vera. *J. Clin. Invest.*, 1930 9 679
17. Henderson, J. L., Blood. A Study in General Physiology. Yale University Press New Haven, 1928.
18. Wintrobe M. M., The hemoglobin content, volume and thickness of the red blood corpuscles in pernicious anemia and sprue, and the changes associated with liver therapy. *Am. J. M. Sc.*, 1931 181 217
19. Wintrobe, M. M., The volume and hemoglobin content of the red blood corpuscle—simple method of calculation, normal findings and value of such calculations in the anemias. *Am. J. M. Sc.*, 1929 177, 513.
20. Haden, R. L., Accurate criteria for differentiating anemias. *Arch. Int. Med.*, 1923 31, 765
21. Capps J. A. A study of volume index. Observations on the volume of erythrocytes in various diseased conditions. *J. Med. Research* 1903 10, 367
22. Haden, R. L. The volume and hemoglobin content of the erythrocytes in health and disease. *Folia haemat.*, 1925 31, 113
23. Goldhamer S. M., Fritzell, A., Davidson, E., and Steen, C., The clinical value of the uncorrected color index and of cell size in pernicious anemia. *Am. J. M. Sc.*, 1932, 184, 165
24. Haden, R. L., The value of volume index in the diagnosis of pernicious anemia. *J. A. M. A.*, 1924 83 671.
25. Murphy W. P., Lynch, R., and Howard I., The value of determinations of the iron content of the whole blood. *Arch. Int. Med.*, 1931 47, 883
26. Reich, C., and Tiedemann, V. G., A study of "Iron volume index" of the blood and its significance in the treatment of anemia. *Am. J. M. Sc.*, 1932, 184 637

- 27 Moore, C, and Doan, C A., The mechanism of post-splenectomy erythroid reëquilibration J A M A. (Proc. Cent. Soc. Clin. Res), 1936, 106, 325
- 28 Murphy, W P, Production of reticulocytes, erythrocytes and hemoglobin in anemia. Arch. Int Med, 1933, 52, 829
- 29 Minot, G R., Cohn, E. J, Murphy, W P, and Lawson, H A., Treatment of pernicious anemia with liver extract. Am. J M Sc, 1928, 175, 599
- 30 Bethell, F H, and Goldhamer, S M, Standards for maximum reticulocyte values following ventriculin and intravenous liver extract therapy in pernicious anemia. Am. J M Sc., 1933, 186, 480
- 31 Isaacs, R., and Friedman, A., Standards for maximum reticulocyte percentage after intramuscular liver therapy in pernicious anemia. Am J M Sc., 1938, 196, 718
32. Reports of the Council on Pharmacy and Chemistry Standardization and labelling of liver and stomach preparations for use in the treatment of pernicious anemia. J A. M A., 1935, 105, 1269

A SECRETORY DEPRESSANT IN GASTRIC JUICE OF PATIENTS WITH PERNICIOUS ANEMIA¹

BY ALEXANDER BRUNSCHWIG JOHN VAN PROHASKA, T HOWARD CLARKE,² AND ERNESTINE KANDEL

(From the Departments of Surgery and Medicine and the Douglas Smith Foundation, The University of Chicago Chicago)

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It has been generally inferred that the achlorhydria and marked gastric hyposecretion, which almost invariably accompany pernicious anemia, are the result of a marked atrophy of the gastric mucosa, the latter change being a part of the classical manifestations of the disease. Achlorhydria of course is not pathognomonic of any specific condition but is observed accompanying a variety of local and systemic diseases, is observed not infrequently in old age and in young and middle aged subjects presenting no other apparent abnormalities. The question of achlorhydria is directly associated with the question of the mechanism of free acid secretion in normal gastric juice. No adequate explanation of this latter mechanism has as yet been made. The commonly accepted theory is that HCl is secreted by the gastric gland cells (parietal cells) in some combined form and that it is not liberated as free HCl until it reaches the foveolae of the glands. The manner of this liberation is likewise not understood. Thus, several possibilities exist to explain any given instance of achlorhydria. There may be a congenital defect in the secretory mechanism of the parietal cells, an atrophy of the fundic glands due to age, or to a variety of pathological conditions or there may be some disturbance in the mechanism for liberation of the bound HCl in the foveolae.

A normal physiological mechanism for the inhibition of gastric secretion has been known for many years starting with the observation that a fatty meal inhibited such secretion as well as gas

tritic motility. This question was studied in greater detail recently by Ivy and his associates. (1) Kosaka and Lim (2) in 1930 showed that this inhibition was accomplished by the production of a hormone (chalone) in the mucosa of the upper small bowel when this was in contact with fat (olive oil). This hormone, which they call "enterogastrone," when injected intravenously in dogs induces a marked inhibition of gastric secretion both as to volume and acid content.

The possibility that achlorhydria in pernicious anemia might be in some way associated with a factor other than simple atrophy of the gastric mucosa does not seem to have been previously investigated. It may be that some substance is formed incident to the development of the disease, which, acting at least in the foveolar portions of the gastric glands or upon the parietal cells themselves leads to inhibition of secretion of HCl and its liberation as free HCl, but this possibility apparently has not received attention. In order to investigate this question it was decided to inject gastric juice from pernicious anemia patients intravenously into dogs with gastric pouches and to observe whether any inhibitory action would result upon the secretion from the pouches stimulated by feeding the animals.

No previous observations have been found in the literature upon the effects of gastric juice itself upon gastric secretion when the former was injected intravenously. Such a study, as regards the stomach at least, would appear justifiable on the principle that one method of studying the pathological physiology of a secreting tissue is to observe the properties of its pathological secretions.

METHODS

Subtotal gastric pouches were made in some dogs by uniting the lower one half of the pylorus to the cardiac portion with closure of the isolated

¹ These observations are incidental to an investigation on the effects of extracts of human gastric cancer on the gastric secretion in dogs which is being carried out under grants from the International Cancer Research Foundation, Philadelphia, Pennsylvania, and the National Advisory Council on Cancer of the U. S. Public Health Service, Washington, D. C.

² Research Assistant, International Cancer Research Foundation Fund.

central portion and cannulation of it with a Dragstedt gold plated cannula which was brought through a stab wound in the abdominal wall. Most of the nerves and arteries to such pouches were preserved. In others, Heidenhain pouches were made and cannulated as just described. Secretion from the cannula was collected in glass Soxlet flasks attached about the body of the animal by cords (See Figure 1)

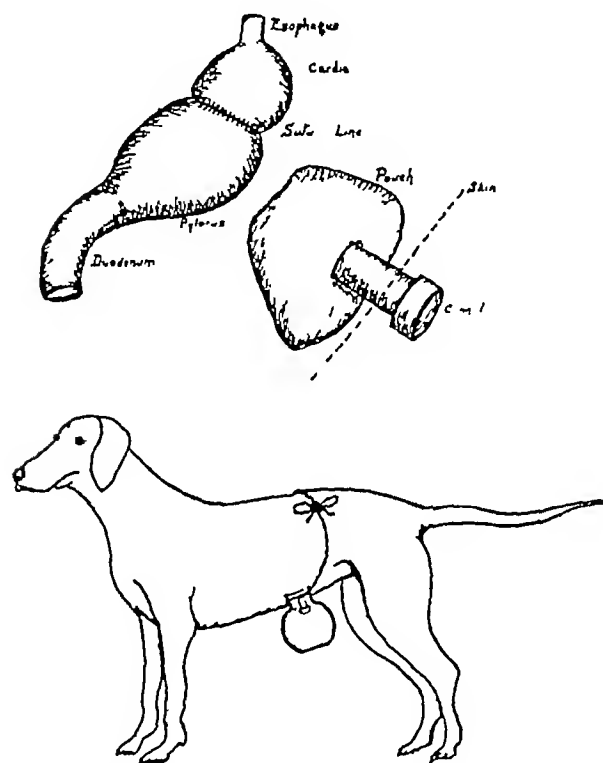


FIG 1 SHOWING METHOD OF COLLECTION OF GASTRIC JUICE FROM DOG WITH SUBTOTAL GASTRIC POUCH

Above, schematic diagram of operation for making gastric pouch. The mid-portion of the stomach is isolated but nerves and vessels to this portion are kept intact. The cardia is anastomosed to the lower one-half to one-third of the pylorus by 2 rows of continuous sutures. The pouch is cannulated through a stab wound in the anterior abdominal wall.

The writers are aware of the criticisms which might be made concerning the secretory activity of gastric pouches, especially of the size of the Heidenhain pouch, namely, that such pouches may not always secrete when the stomach itself is actively secreting. In the following experiments no dog was employed in whom adequate control experiments had not been performed to demonstrate

the reliability of the secretory mechanism of the pouch when the animals were fed.

In this series of experiments interest was centered upon factors inhibiting gastric secretion. Thus at the beginning of the experiment the dogs were fed (forcefully if necessary) cooked and raw meat, or lung stew and were permitted free movement upon a large table with access to limited quantities of water. When active secretion was established in 30 to 60 minutes, the human gastric juice to be tested (previously neutralized with $1/10$ N NaOH if necessary) was injected intravenously in an extremity and the effect noted upon the pouch secretion. The pouch juice was collected at 10-minute intervals and titrated with Toepfer's reagent and phenolphthalein for free and combined acid, the results being expressed in "clinical units." One feeding, *i.e.*, a mass of food totalling approximately 6 to 8 cm in diameter given in smaller boli was sufficient to insure active secretion for over an hour in most dogs. However, to insure adequate stimulation, especially where the experiments were critical, voluntary or forced feedings were given at 20 to 40-minute intervals throughout the experiment. Control experiments showed that once active secretion was established vomiting of all food taken did not result in a sudden arrest of secretion from the pouch but that this often continued for approximately one hour or more if the animal was not refed. In critical experiments, however, the animal was refed from 5 to 10 minutes after any vomiting to insure continued food stimulation of the stomach.

The gastric juices were obtained by tube aspirations from patients who had received subcutaneous injections of histamine, according to the usual technique of a histamine test for gastric secretion. After centrifugation they were neutralized with $1/10$ N NaOH when necessary, the added volume being taken into consideration when injections were made in the animals. These injections were arbitrarily fixed at 1 cc of gastric juice per kilogram of the animals' weight. When possible, juices were injected within a few hours after recovery, otherwise they were kept on ice.

The patients with pernicious anemia were from the Hematology Clinic of the Department of Medicine and were clinically and hematologically typical of pernicious anemia. Hemoglobin deter-

minations were made by the Newcomer hemoglobinometer, and blood counts and cell volumes by the standard methods. All cases showed a macrocytic hyperchromic anemia when first seen. Routine gastro-intestinal x rays were made in all cases to rule out other gastro-intestinal diseases. Histamine tests of gastric secretion showed no free HCl on various occasions. Reticulocyte response to liver therapy was prompt. The control patients were from the various hospital services, all being sufficiently ill to have been hospitalized (i.e. the control group was not made up of normal individuals).

RESULTS

A Preliminary control experiments, 12 in number, were performed in which the neutralized gastric juice from the pouch of a dog fed lean meat was injected intravenously into that dog. No effects were observed upon secretion from the pouch stimulated by feeding. Similar negative results were obtained by injection of neutralized gastric juice from other dogs' pouches.

The effect of temperature. In 1932 Vanzant and Snell (3) observed inhibition of gastric secretion in dogs whose temperature was suddenly elevated by injection of bacterial protein. It was observed by us that a rise in temperature over 30 minutes to 1 or 2 hours to above 40.5° C. (rectal) resulted in a rather sudden and marked depression in secretion of gastric juice and an achlorhydria. Thus rectal temperature readings were taken at intervals of 10 to 30 minutes throughout the experiments and when they rose above 40.5° to 40.6° the experiment was not accepted. The animals were then 'vaccinated' by repeated daily injections against the fever producing elements in the juice. After some time some of the animals became 'temperature resistant' and continued to secrete acid juice in adequate quantities while having temperatures as high as 42° C. Obviously, once such a temperature resistance developed as shown by control experiments results observed during any temperatures up to this point were considered valid in these animals.

In the literature on experimental studies of substances inhibiting gastric secretion no attention seems to have been paid to sudden rises in temperature due to such injections.

B The intravenous injection of achlorhydric gastric juice from patients with pernicious anemia.

The results observed upon stimulated pouch secretions are given in Table I and Figure 2. The result recorded opposite the abbreviated name of the animal employed indicates one acceptable experiment.

The secretory inhibition was manifested in most of the positive experiments after a latent period of 20 to 60 minutes following injection, but in some cases it was as long as 90 to 120 minutes.

Cases 1 to 5 inclusive were of long standing and had had blood counts that were normal or above normal, with slight lapses during intercurrent infections, for a number of years.

TABLE I
Summary of experiments with gastric juice from patients with pernicious anemia

Patient and history number	Max. mm free HCl in gastric juice (Hista mine test)	Inhibition of secretion from stimulated pouch *	Effect of juice boiled for 10 minutes
1. Jurg (22567)	0	Dog Trix Nap +++++	Dog Nap —
2. Sim (23156)	0	Bud +++++	Bud —
3. Thom (78289)	0	Trix +++++ Trix — Kg +++ Nap +	
4. Mol (132207)	0	Kg +++	Kg —
5. Pir (128721)	0	Bud +++++ Bud +++++ Bud +++++	Bud —
6. Sul (203092)	0	Kg — Fid +++++ May + May — Nap —	
7. Gre. (195942)	0	Trix +++++ Kg — Kg ++ May +++ Nap —	May —
8. Hans (198755)	0	Mon — Mon. —	
9. Tub (206430)	0	Kg +++++ Nap ++	Kg —
10. Conl (196359)	0	Mon +++++ Nap — Sam. +++++	Mon —

TABLE I—*Continued*

Patient and history number	Maximum free HCl in gastric juice Hista mine test	Inhibition of secretion from stimulated pouch *	Effect of juice boiled for 10 minutes
11 Lit † (200796)	0	Fid +++++ Kg +++++	
12 Hurb (20331)	0	Kg — May — Maz —	
13 Lars (205015)	0	Kg — Nap +	
14 Gold (205946)	0	Nap — Nap + Nap +++++ Nap +(?)	Nap —
15 Dalt	0	Heavy +++++ Sam +++++ Heavy +++++	
16 Dannen	0	Sam +++++	Sam —
17 Schieff	0	Heavy +++++ Heavy +++++ Sam —	
18 Hathaway	0	Spoof +++++ Heavy +++++	

† Patient also had carcinoma of the stomach

* — = No effect on secretion of stimulated pouch

+ = Marked reduction in volume of secretion per 10-minute period to 4 drops or less for at least 2 such periods and with persistence of free acid or reduction in free acid titer to 25 or less clinical units providing the secreted juice exhibited at least 60 or 70 clinical units before injection

++ = Reduction in volume of secretion with actual achlorhydria for at least 10 minutes but not more than 30 minutes

+++ = Reduction in volume of secretion with actual achlorhydria for 30 to 60 minutes

++++ = Reduction in volume of secretion with actual achlorhydria for 60 minutes or longer

Cases 6 to 11 inclusive had had several years of irregular therapy before being seen in this clinic, and when gastric juices were aspirated for these experiments the red cell counts were low. Case 11 also had a small carcinoma of the pylorus which was resected.

Cases 12 to 14 inclusive were admitted to the hospital with very low counts and had had no previous liver therapy. Case 13 secreted very little juice in spite of histamine injection. The negative response observed in Dog Kg was obtained when the patient was still very weak and yielded only enough juice to equal 80 per cent (35 cc) of the regular dose for the dog. The

one plus result observed in Dog Nap was obtained some time later when the patient had improved clinically but was still unable to yield more than 75 cc. (about half the necessary dose for this dog) of juice during 80 minutes following histamine injection. A third attempt after further clinical improvement yielded only 1 cc of gastric juice, insufficient for an experiment. Case 14 was also very weak on admission and the first aspiration, obtained with difficulty, gave the negative result. The subsequent aspirations which gave positive results were made when there had been improvement in the blood and general condition.

Case 15 had a walnut-sized benign papilloma of the stomach which was successfully excised.

Case 16 had had a radical gastrectomy 5 years previously for carcinoma and when seen at the time of this experiment had typical pernicious anemia without evidence of recurrent carcinoma.

Case 18 also presented hyperthyroidism which was successfully treated by operation.

It is to be noted that the dogs vary widely in the quantity of secretion produced by the pouch depending upon its size and size of the dog. Also, dogs vary in their response to factors depressing secretion, some "weak secretors" responded markedly to the depressing factor, other "strong secretors" were not as easily rendered achlorhydric, and when this did develop it was relatively less prolonged. Furthermore, the dogs may be strong secretors on some days and weaker secretors on other days. These characteristics became evident during a number of experiments upon each dog. Marked variations in volume of secretion such as a drop from 3 cc or more to 0.4 or 0.5 cc per 10-minute period following injection were ignored. Furthermore, variations in the titer of free acid of the order of 50 or 60 or more clinical units were also ignored where such titers did not fall below 30 units. (For example, a transitory drop in titer of free acid from 120 to 40 or 50 after injection of a pernicious anemia gastric juice was still considered a negative response.)

An endeavor was made to obtain more than one acceptable experiment with each sample. However, this was not always possible as much of a sample might be used in experiments that could not be accepted because the achlorhydria was accompanied by hyperthermia, and so additional

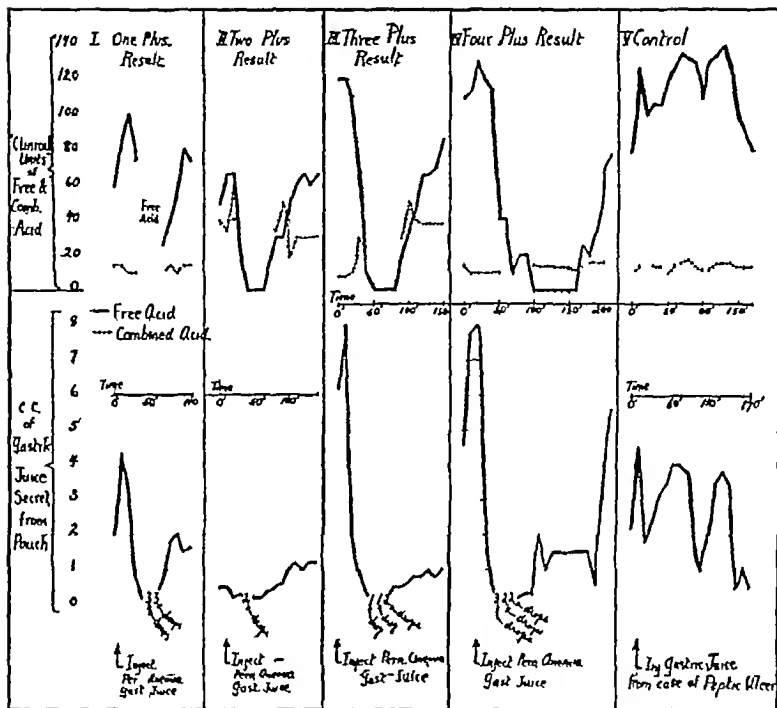


FIG. 2. CONDENSED GRAPHS TO SHOW EFFECTS OF INJECTION OF GASTRIC JUICES FROM PATIENTS WITH PERNICIOUS ANEMIA ON SECRETIONS FROM STIMULATED GASTRIC POUCHES IN DOGS

I A one plus result, the volume of secretion is reduced to 0.2 cc. 3 and 3 drops respectively for each of 3 10-minute periods. Free acid always present.

II A two plus reaction, reduction in volume of secretion with achlorhydria for 30 minutes.

III A three plus reaction achlorhydria for 40 minutes with reduction in volume of secretion.

IV A four plus reaction, achlorhydria for 70 minutes with reduction in volume of secretion.

V A negative response in a control experiment in which neutralized gastric juice from a patient with peptic ulcer was injected.

quantities of the sample had to be employed for immunization of the dog by repeated small injections against the fever producing substances in the juice.

While it was impractical to carry out all positive and negative experiments in the pernicious anemia and in the control group in each dog all of the dogs in the pernicious anemia group of experiments were also employed in several of the control experiments

Boiling the juices for 10 minutes inactivated the gastric secretory depressant if the latter was present in the unboiled sample

C Control experiments with human gastric juice. Gastric juices from 34 patients not suffering from pernicious anemia or malignant neoplasm were also obtained according to the usual procedure for the histamine test for gastric secretion and these were injected (after neutralization if

necessary) into the dogs as controls for the experiments in Group B. The results are summarized in Table II. In 6 out of 34 instances inhibition of pouch secretion was noted (Cases 1, 13, 19, 26, 30, 32) and, as in the previous group, except for one instance, boiling these "positive" samples resulted in inactivation of the secretory inhibitor. Fourteen of the control group yielded achlorhydric gastric juices following histamine injection and, of these, 2 samples afforded positive experiments. Cases 27 and 28 were artificial achlorhydrias having been produced some time previously by radiation of the stomach with x-ray in the treatment of peptic ulcer.

DISCUSSION

Samples of gastric juices from 16 of 18 patients with pernicious anemia, 89 per cent of the samples,

TABLE II

Gastric juices with normal or high acid from patients not presenting malignant neoplasms or pernicious anemia

Patient	Diagnosis	Maximum free acid (histamine)	Dog and result	Boiled
1 K	Acute cellulitis of legs	80	Trix Bud Fld. ++++	Trix Bud -
2. Feh.	Millary tuberculosis	75	Min Trix -	
3. Thomp.	Hypertrophic gastritis	45	Nap Kg. -	
4. Ojal.	Peptic ulcer	40	Nap Nap -	
5. Stud.	Peptic ulcer	70	Tr -	
6. Gorm.	Peptic ulcer	65	Tr -	
7. Bur.	Peptic ulcer	40	Min. -	
8. Wurth.	Peptic ulcer	80	Kg. -	
9. Wolff	Peptic ulcer	100	Nap Nap -	
10. Bergst.	Peptic ulcer	68	Nap Kg. -	
11. Savage	Tubercular lymphadenitis	20	Nap -	
12. Fetter	Secondary anemia	75	Nap -	
13. O'Dell.	Cholecystitis	80	Heavy Eva Dan Heavy ++++	Heavy ++++
	2d specimen	30	Heavy ++	
14. Senetps	Lues	65	Heavy Dan -	
15. Shulman.	Hypothyroidism	76	Heavy Bust. -	
16. Johnson	Cholecystitis	60	Heavy Dan -	

TABLE II—Continued

Patient	Diagnosis	Maximum free acid (histamine)	Dog and result	Boiled
17. De Chat	Pancreatitis	65	Dan Bust -	
18. Furman	Peptic ulcer	70	Heavy -	
19. Booga	Cholecystitis	75	Droopy Spoof Lix. - +	
20. Barry	Cholecystitis	15	Jok -	
21. Furl	Cholecystitis	0	Monk -	
22. Roap.	Senility	0	Kg. -	
23. O'H	Osteoporosis	0	Kg. -	
24. Finl.	Chronic nephritis	0	Nap Kg. -	
25. Rhode	Peptic ulcer	0	Kg. Nap -	
26. Child.	Peptic ulcer	0	Min Min ++	May -
27. Fred.	X-ray achlorhydria	0	Sk -	
28. Bath	X-ray achlorhydria	0	Monk -	
29. Gesth.	Cholecystitis	0	Eva Heavy Dan -	
30. Murry	Cirrhosis	0	Dan Heavy ++ + + +	Dan -
31. Kinoski	Peptic ulcer	0	Nap Nap -	
32. Debris	Cirrhosis	0	Heavy Dan +	
33. Laws	Hyperthyroidism	0	Bust -	
34. Ofel	Neuritis	0	Heavy -	

when injected intravenously in dogs with stimulated gastric pouches resulted in marked inhibition of pouch secretion and achlorhydria. Gastric juices from 6 of 34 patients, 18 per cent of the samples, not suffering from pernicious anemia or malignant neoplasms produced similar effects. While the incidence of gastric secretory depression is much greater in the former than the latter group, other possibilities must be considered before assuming that it is due to a factor preponderant in pernicious anemia gastric juice.

Age of the patient No correlation between the age of the patient and depressing effects of the injected gastric juice was possible.

Temperature The effect of temperature upon gastric secretion in the dogs has been discussed above and ruled out as a causative factor in the positive experiments obtained from injection of gastric juice from control or pernicious anemia patients.

Blood pressure It is conceivable that a marked splanchnic vasodilation would result first in a stimulation of gastric secretion and then if this persisted a reduction in secretion. Experiments were performed upon anesthetized dogs whose carotid arteries were cannulated to a manometer and blood pressure variations observed following intravenous injection of "positive" pernicious anemia juices saliva from pernicious anemia patients and control human gastric juices. A wide variation in effects upon blood pressure occurred, but it was clearly shown that the pernicious anemia gastric juices which produced marked inhibition of gastric secretion in dogs with gastric pouches did not produce significant blood pressure changes.

Carbon dioxide combining power of the blood According to Browne and Vineberg (4) when the carbon dioxide combining power of the blood of dogs falls to 30 volumes per cent or less, gastric secretion is inhibited. In two dogs during achlorhydric periods resulting from injection of gastric juice from pernicious anemia patients, samples of blood withdrawn showed no reduction in CO_2 combining power. When blood was withdrawn from dogs showing achlorhydria due to injection of enterogastrone (see below) the CO_2 combining power was found not to be lowered when compared with samples withdrawn before the injection when acid juice was being secreted by the pouch.

Saliva. Saliva was collected from 5 of the patients with pernicious anemia whose gastric juices were tested above, by having them chew paraffin and expectorate into receptacles. Each of the samples was injected intravenously into a dog under the same conditions as in the injection of gastric juice a total of 7 experiments being performed. In no instance was an achlorhydria produced. In 2 experiments the pouch secretion volume fell to 3 drops for one 10-minute period, 80 minutes after injection.

Enterogastrone As stated above Ivy and Gray (1) and Kosaka and Lam (2) showed that intravenous injection of extracts of duodenal mucosae that have been in contact with fat depress gastric secretion in the dog. The factor, a chalone, responsible for this has been called enterogastrone. As far as the writers have been able to determine, no investigations have been previously reported in which gastric juice was examined for enterogastrone. In two experiments,

the injection of an achlorhydric dog's pouch juice into other dogs failed to suppress secretion. The achlorhydric juice in these instances was obtained in one case as a result of induced hyperthermia and in the other it was obtained from a pouch which did not secrete acid although the dog was fed lean meat. In other dogs with achlorhydria due to injection of enterogastrone, insufficient quantities of the achlorhydric juice were obtained to perform injections in doses similar to those employed in the experiments with human juice. In a further attempt to obtain pouch juice that might contain a depressant, dogs were fed a small piece of lean meat and then at 10 to 20-minute intervals they were fed raw and warmed suet, some of which was also triturated with olive oil. In this manner the gastric and duodenal mucosa were brought into contact with fat and conditions made favorable for generation of enterogastrone. Under these conditions the pouch secretions were less in volume than if the dogs had been fed lean meat. However, marked reduction in free acid titer of the juices was not regularly observed. Commencing one hour after the beginning of the fat feedings the samples of juice obtained during the next 2 to 3 hours were pooled, neutralized, and re-injected the following day into the dogs which, however, were now fed lean meat. Of 10 such experiments 8 were negative, 1 was considered a + experiment and 1 a ++ result. Comparing these results with the 12 negative control experiments of Group A, it was concluded that under the conditions of the experiments enterogastrone generated in a physiological manner might appear only in occasional instances in sufficient concentration in dogs' gastric juice to suppress gastric pouch secretion when the juice is injected in doses of 1 cc of the "enterogastrone juice" per kilogram weight of the animal.

The question then arising is whether the gastric secretory depressant observed in the experiments with human juice might not be due to an abnormally large amount of enterogastrone. Against such an explanation is the fact that in the patients the gastric juice was collected in the morning after several hours starvation and upon histamine stimulation, and thus there was no opportunity for recent or prolonged contact of the duodenal mucosa with fat. This would favor the view that the secretory depressant in human gas-

tric juices might be some factor other than enterogastrone, although of similar physiological action. On the other hand, the incidence of positive experiments among the controls is, in our opinion, too high to consider merely as "false positives" and would suggest that the gastric secretory depressant found apparently in relatively high concentration in pernicious anemia juice represents a marked increase of a factor that may be normally present in relatively low concentrations (enterogastrone?) in samples of gastric juice from patients not having pernicious anemia. The differentiation of enterogastrone from other possible secretory depressants in gastric juice will be dealt with in a subsequent publication. Furthermore, studies similar to those carried out above but with achlorhydric juices obtained from patients with gastric carcinoma and malignant neoplasms outside the stomach will also be reported.

SUMMARY

The gastric juices from 16 of 18 patients with pernicious anemia and achlorhydria, when injected intravenously into dogs with gastric pouches, the secretion of which had been stimulated by feeding, resulted in a transitory marked depression

of the pouch secretion and achlorhydria (89 per cent of the samples). The injection of gastric juices from 34 hospitalized patients not having pernicious anemia or malignant neoplasms yielded similar gastric secretory inhibition in 6 instances (18 per cent of the samples). The secretory depressant effect of a sample of human gastric juice was abolished by boiling for 10 minutes.

The hypothesis that achlorhydria, in pernicious anemia at least, is associated with the presence of some gastric secretory inhibitor or to some profound distortion of normal hormonal control of gastric function is discussed.

BIBLIOGRAPHY

- 1 Ivy, A. C., and Gray, J. S., Enterogastrone. Published in Cold Spring Harbor Symposia on Quantitative Biology, 1937, 5, 405.
- 2 Kosaka, T., and Lim, R. K. S., Demonstration of the hormonal agent in fat inhibition of gastric secretion. *Proc. Soc. Exper. Biol. and Med.*, 1930, 27, 890.
- 3 Vanzant, F. R., and Snell, A. M., The effect of non-specific protein on the pain of ulcer and on gastric secretion. *J. Clin. Invest.*, 1932, 11, 647.
- 4 Browne, J. S. L., and Vineberg, A. M., The interdependence of gastric secretion and the CO_2 content of the blood. *J. Physiol.*, 1932, 75, 345.

SULFAPYRIDINE, SULFANILAMIDE AND SPECIFIC ANTISERUM IN EXPERIMENTAL TYPE III PNEUMOCOCCIC INFECTIONS

By FRANK B COOPER, PAUL GROSS, AND MARION LEWIS

(From The Western Pennsylvania Hospital Institute of Pathology Pittsburgh)

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It has been claimed by Whitby (1) that sulfapyridine saved a majority of mice infected intraperitoneally with 10,000 fatal doses of pneumococci of Types I, II, III, V, VII, and VIII, its therapeutic efficacy being more pronounced against Types I, VII and VIII. Furthermore, mice which recovered by virtue of this presumably non-toxic drug were immune to a second infection of 10 000 and in some instances 1 000 000 fatal doses of pneumococci as early as the end of the first week.

The announcement of this experimental work was quickly followed by the clinical report of Evans and Gasford (2) who claimed a reduction in mortality from 27 in 100 untreated cases of pneumonia to 8 in 100 who received sulfapyridine. These two papers are responsible for the present intense interest in sulfapyridine as an antipneumococcal drug.

A comparison of the efficacy of sulfapyridine and sulfanilamide by Cooper, Gross, and Lewis (3) against Type II pneumococcal infections of less than 100 fatal doses in both mice and rats showed the former compound slightly more effective than the latter, although approximately one-half of the animals in the treated groups died.

In a simultaneous publication Hilles and Schmidt (4) reported sulfapyridine not significantly superior to sulfanilamide against mouse infections of 100 fatal doses of Type XXII pneumococci. Unfortunately, the results obtained are not comparable because different dosages of the two drugs were administered by two different methods. Although sulfapyridine prolonged the lives of all 20 mice 9 living 6 days or longer, all but one died before the fifteenth day. Similarly in the sulfanilamide group most of the mice died after the sixth day and all by the thirteenth day. In a subsequent experiment, 14 of 20 mice which received 80 mgm of sulfapyridine orally each day for 4 days then 40 mgm. daily for 2 days, survived 30 days. A comparable sulfanilamide experiment was not recorded.

Long, Bliss and Feinstone (5), on the other hand concluded that sulfapyridine was considerably more effective than sulfanilamide in the treatment of experimental Type I infections of mice. These conclusions were based on a 12 per cent survival in the sulfapyridine group and no survivors in the sulfanilamide group, both of which were infected with approximately 1180 fatal doses.

Because of the lack of agreement between Whitby's (1) and our own (3) results, experimental work has been continued and the comparative efficacy of sulfapyridine, sulfanilamide, and specific antipneumococcal rabbit serum determined against Type III pneumococcal meningitis and pneumonia of rats as well as septicemia of mice.

The immunity of the recovered animals from this and from a preceding experiment (3) was determined by the intraperitoneal inoculation of 1 and also of 100 fatal doses of homologous culture.

The purpose of the following report is to present the data from this study.

Pneumococcal meningitis of rats

Method Six groups of 15 rats each were infected intracranially (3 6 7 8) with a suitable dilution of a broth culture of Strain 420 which was shown by previous and simultaneous intracranial titration to contain 10 fatal doses. This culture was selected because it typed well with therapeutic Type III rabbit serum¹ and with Type III typing serum but did not cross type with Type VIII serum.

One group served as untreated controls, while the remaining 5 were treated, as shown in Figure 1, with sulfanilamide,² sulfapyridine,³ Type III antipneumococcal rabbit serum, sulfanilamide plus serum, and sulfapyridine plus serum.

¹ Kindly donated by E. R. Squibb & Sons, New York.

² Synthesized and donated by the Monsanto Chemical Co., St. Louis, Mo.

³ Supplied by Merck & Co., Inc., Rahway N J.

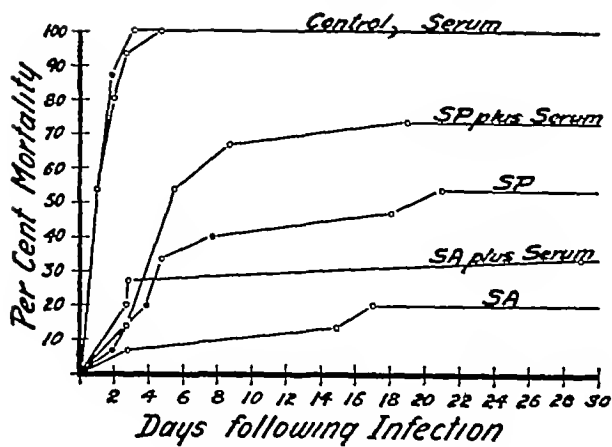


FIG 1 MENINGITIS

Mortality curves of rats infected with 10 fatal doses of Type III (Strain 420) pneumococcus and treated 6 hours after infection

Infection 0.1 cc. of a 10^{-4} broth dilution of an 18-hour broth culture intracranially

Treatment Controls 15 rats, no treatment.

SA 15 rats, 100 mgm. of sulfanilamide in 0.5 cc. of 15 per cent gum acacia orally 6 hours after infection, then twice daily for 7 days, followed by 100 mgm once daily for 7 days

SP 15 rats, same dosage of sulfapyridine.

Serum 15 rats, 333 units of Type III rabbit antipneumococcal serum intraperitoneally 6 hours after infection, then once daily for 2 successive days (1000 units)

SA plus serum 15 rats, combination of sulfanilamide and serum therapy used above.

SP plus serum 15 rats, combination of sulfapyridine and serum therapy used above.

The immunity of the rats which survived for one month was determined by infecting them intraperitoneally with approximately one fatal dose, determined by intraperitoneal titration, of the same Type III strain

Results As shown in Figure 1, all untreated rats died in 2 days and all serum-treated rats within 5 days. The sulfanilamide- and sulfapyridine-treated groups suffered a mortality of 3 and 8, the sulfanilamide plus serum and sulfapyridine plus serum groups, 5 and 11 of 15 rats respectively. It is significant that of the 75 rats which received some form of treatment, 6 died late in the experiment of a relatively fresh meningitis

Of the 32 rats which recovered from meningitis as a result of treatment, 22 failed to survive the intraperitoneal infection of approximately one fatal dose of the same strain of Type III pneumococci one month later. The distribution of

casualties in this group was as follows: sulfanilamide, 7 of 11; sulfapyridine, 4 of 7; sulfanilamide plus serum, 8 of 10; and sulfapyridine plus serum, 3 of 4. These results are comparable to the mortality rate of 7 of the control group of 10 normal rats. Similarly, all 33 survivors from an earlier Type II meningitis experiment (3) died when reinfected, this time intraperitoneally, with less than 10 fatal doses of homologous culture. This infecting dose killed 9 of 10 normal rats.

Pneumococcal pneumonia of rats

Method Four groups, each of 15 rats, were infected intratracheally, as described in previous experiments (9, 10, 11, 12) with a mucin suspension of approximately 100 fatal doses of Strain 420 Type III pneumococci. One group received no treatment, whereas the remaining 3 groups were treated with sulfanilamide, sulfapyridine, and Type III antipneumococcal rabbit serum as shown in Figure 2.

Results Reference to Figure 2 shows that all

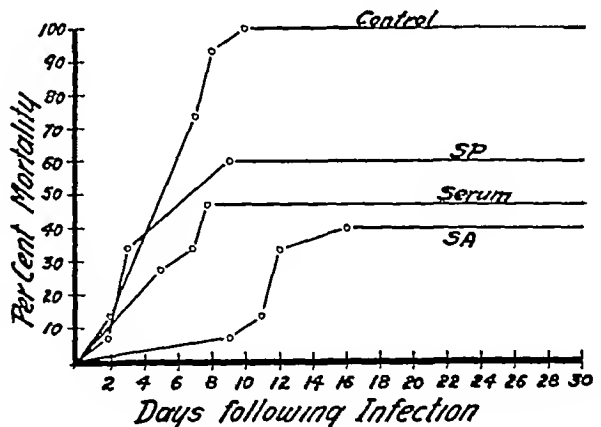


FIG 2. PNEUMONIA

Mortality curves of rats infected with 100 fatal doses of Type III (Strain 420) pneumococcus and treated 5 hours after infection.

Infection 0.15 cc. of a mucin suspension of an 18-hour broth culture, diluted 10^{-1} intratracheally

Treatment Controls 15 rats, no treatment.

SA 15 rats, 100 mgm. of sulfanilamide in 0.5 cc. of 15 per cent gum acacia orally 5, 12, and 21 hours after infection, then twice daily for 5 days, followed by 100 mgm once daily for 3 days.

SP 15 rats, same dosage of sulfapyridine.

Serum 15 rats, 333 units of Type III rabbit antipneumococcal serum intraperitoneally 5 hours, 1, and 2 days after infection and 500 units on the third day (1500 units)

control rats died in 2 to 10 days after infection, whereas the mortality of the sulfanilamide, sulfapyridine, and serum treated groups was 6, 9, and 7 of 15 respectively one month later. All fatalities, except four in the sulfapyridine group showed at autopsy the type of experimental pneumonia previously described.

Bacteremia as demonstrated by culture from the femoral vein at autopsy, was present in all control and sulfanilamide treated rats, but was absent in several serum treated rats and several treated with sulfapyridine.

In this experiment, as in the preceding one, there was a number of delayed deaths 5 of which occurred in the sulfanilamide group from 11 to 16 days and one in the sulfapyridine group 9 days after infection.

The immunity of the pneumonia survivors was considerably greater than that of the meningitis survivors since there were only 4 deaths out of 18 survivors distributed as follows: 1 of 8 in the sulfanilamide, 3 of 4 in the sulfapyridine, and none of 6 in the serum group whereas 11 of 14 normal control rats died.

Pneumococcus septicemia of mice

Method Four groups, each of 10 mice, were infected subcutaneously with more than 100 fatal doses of the same 420 strain. (Titration mice which received $\frac{1}{10}$ and $\frac{1}{100}$ of the infecting dose used in the experiment died within 72 hours.) One group served as untreated controls, while the remaining groups were treated as shown in Table I.

Results The mortality rates of the various groups were: controls 10, sulfanilamide, 9, sulfapyridine, 5, and serum 5 of 10 mice.

Since the number of survivors from this experiment was too small to be statistically significant the survivors from some unreported mouse experiments were reinfected intraperitoneally with 100 fatal doses of the homologous Type II (Binda) culture. Reference to Table II indicates the presence of some degree of immunity in the recovered mice.

DISCUSSION

The above experiments show that equal doses of sulfanilamide or sulfapyridine possess approxi-

TABLE I

*Therapeutic efficacy of sulfanilamide, sulfapyridine, and specific antipneumococcal serum against Type III pneumococcal infection of mice**

Treatment†	Number of mice	Number of deaths daily during 21 days						Number of fatalities
		1	2	3	4	5	6-7-13	
None	10		3	6	1			10
Sulfanilamide	10	1		1	2		3	9
Sulfapyridine	10				1	1	3	5
Type III rabbit serum	10	1	2	2				5

* Infection 0.5 cc. of a 10^{-7} broth dilution of an 18-hour broth culture (100 fatal doses) of Type III (Strain 420) pneumococcus subcutaneously.

† Treatment: Sulfanilamide 20 mgm. of sulfanilamide in 0.2 cc. of 15 per cent gum acacia 3 hours after infection, then once daily for 8 days.

Sulfapyridine: Same dosage as of sulfanilamide.

Serum: 100 units of Type III antipneumococcal rabbit serum intraperitoneally 3, 24 and 48 hours after infection (300 units).

TABLE II

*Immunity of recovered mice to reinfection with 100 fatal doses of homologous culture**

Previous treatment	Number of mice	Number of survivors
None	26	None
Sulfanilamide	9	7
Sulfapyridine	18	6

* Infection 0.5 cc. of a 10^{-1} broth dilution of an 18-hour broth culture of homologous Type II (Binda) culture (100 fatal doses).

mately the same therapeutic efficacy against both Type III pneumococcal meningitis and Type III pneumococcal pneumonia of rats. An earlier experiment also showed these drugs to be of approximately equal value in treating experimental Type II pneumococcal meningitis (3). In mice, however, sulfapyridine was superior to sulfanilamide against Type III pneumococcal sepsis in the above experiment as well as Type II sepsis in the previous experiment (3). This is not surprising since the computed efficacies of any 2 drugs which possess approximately the same value may vary somewhat in either direction when different types and strains of pneumococci or animals are used. Although variations of this kind have been attributed to strain (13, 14) and to type (4) differences both the experimental work and the cures of clinical pneumococcal meningitis point to strain difference as the more important factor. This view is strengthened by our experience with

3 Type II strains which show marked differences in invasive power and in response to chemotherapy. However, experimental results show that neither sulfapyridine nor sulfanilamide is effective against pneumococcal infections of mice or rats when the infection exceeds 100 fatal doses. This statement receives added support from the results of experiments (15) in which mice were infected with approximately 100 lethal doses of Type II pneumococci and treated 4 hours later. An aggregate of 80 mice treated with sulfapyridine suffered a 55 per cent mortality while an equal number of mice treated with an equal dosage of sulfanilamide showed a 74 per cent mortality at the end of 3 weeks. Hilles and Schmidt (4) obtained similar results in mice treated with 4 grams per kilo the first day, followed by 5 daily treatments of 1 gram per kilo, but saved 14 out of 20 mice when 4 grams per kilo were given for 4 days and 2 grams per kilo for 2 additional days.

In view of the discrepancy between the results so far reported and Whitby's (1) claim that sulfapyridine saved a majority of mice infected with 10,000 fatal doses of pneumococci, a further consideration of the method whereby such favorable results were obtained appears indicated. In Whitby's various publications (1, 16, 17), the therapeutic efficiency of each drug under investigation was expressed by a figure designated as the "survival value." This figure represented the average survival time of groups of mice, usually 6 in number, which were observed in most instances for 7 days. This simple method of evaluation is open to question since it ignores ultimate survivals and may give the same value, such as 5 out of a possible 7, in experiments which produce quite different end results. For example, a value of 5 would be obtained in an experiment in which one-half of the mice survived indefinitely, while 1 of each of the remaining 3 died at the end of the second, third, and fourth days. Similarly, a value of 5 would be obtained if all mice died at the end of the fifth day or if 2 died at the end of each of the fourth, fifth, and sixth days, leaving in the last 2 instances no survivors. The 7-day period of observation, considered adequate by Whitby (1, 16, 17, 18) was found to be insufficient by others who observed a significant number of deaths between the second and fourth weeks of observation (3, 4, 9, 10, 12, 19, 20, 21).

Another circumstance which throws doubt upon the validity of this method of evaluation is the difference in values which the author reported for sulfanilamide against Type I infections: 1.2 out of a possible 7 in 1937 (18) and 3.3 out of 7 in 1938 (1).

It is evident from Experiment I that sulfanilamide and sulfapyridine are about equally effective in treating Type III experimental meningitis, whereas Type III rabbit antipneumococcal serum was valueless. However, in Experiment II all three medications appeared equally effective, in the dosages employed, against pneumococcal pneumonia. This latter observation is confirmatory of previous conclusions concerning the therapeutic value of sulfanilamide and specific antiserum in experimental Type I (10) and Type II (12) pneumococcal pneumonia and Type I pneumococcal meningitis (8).

The serum dosage was calculated on a weight for weight basis from a human dosage of 350,000 units for Experiment I, one and one-half times that amount for Experiment II, and three times the amount for Experiment III.

The dosage of sulfanilamide employed throughout was that which has consistently given good results in our hands (6, 7, 9, 10, 11, 12). The fact that this dosage of 0.5 to 1.0 gram per kilo of rat is, weight for weight, considerably more than the 0.08 to 0.15 gram per kilo generally advocated for man (22), has occasionally prevented the casual observer from recognizing the clinical application of the animal experiments. For example, the Pneumonia Commission of the Medical Society of the State of Pennsylvania (23) was of the opinion that the amount of sulfanilamide required for adequate treatment of clinical pneumonia, calculated from the quantity used in animals, was so great as to be dangerous. Marshall and Cutting (24) have subsequently shown such fears to be groundless since only a fraction of the dose which is required to maintain the therapeutically effective concentration of 5 to 15 mgm per cent in the rat is necessary to produce the same optimum blood level in man. In the mouse, however, 1.0 gram per kilo produced the high concentration of approximately 50 mgm per cent during the first 2 hours, 20 to 25 mgm per cent by the sixth hour and about 6 mgm per cent at the end of 24 hours. A dose of 0.4 gram per

kilo gave a maximum concentration of 20 mgm per cent the first hour but a concentration of only 4 mgm per cent within 6 hours (24). From this it is evident that the rat is the experimental animal of choice, not only because of the ease in producing the pneumococcal diseases most frequently encountered in humans, namely pneumonia and meningitis, but because daily or twice daily treatments produce blood concentrations of sulfanilamide of the same order as those considered optimum in clinical practice. Furthermore, the validity of the results obtained experimentally in rats is being substantiated by the rapidly accumulating reports of clinical cures of both pneumococcal pneumonia and meningitis (8).

The high degree of immunity which Whithy (1) claimed followed the recovery of sulfapyridine-treated mice was absent in our series of meningitis and pneumonia rats which received sulfapyridine. In fact, these rats showed numerically less immunity than those treated with sulfanilamide or serum when reinjected with one or ten carefully titrated fatal doses of homologous culture. It would also appear that late deaths would have been less frequent in both series if a significant degree of immunity had followed treatment and apparent recovery. In regard to the immunity of recovered mice, Fernstone *et al* (25) observed none in those which had received 44'-diaminodiphenylsulfone, when reinjected 30 days later with 10 to 100 fatal doses. Although our mice showed a certain degree of immunity it did not approach that claimed by Whithy (1) since 12 of 18 sulfapyridine recoveries and 2 of 9 sulfanilamide recoveries died when reinjected with only 100 fatal doses.

CONCLUSIONS

1 Sulfanilamide and sulfapyridine⁴ were equally effective against Type III experimental pneumococcal meningitis and pneumonia of rats, whereas

⁴While this paper was in press Gross, Cooper and Lewis (Proc. Soc. Exper. Biol. and Med. 1939 40, 448) and Antopol and Robinson (Ibid., 1939 40 428) simultaneously reported kidney damage due to acetyl-sulfapyridine uroliths in rats fed sulfapyridine. If it were possible to obviate the renal complications in rats treated with sulfapyridine, it would seem likely that the latter drug would prove to be slightly superior to sulfanilamide in the rat as well as in the mouse.

sulfapyridine was somewhat superior against Type III pneumococcal sepsis of mice. Neither drug was effective against more than 100 fatal doses.

2 Specific Type III pneumococcal rabbit antiserum was as effective as sulfanilamide or sulfapyridine in Type III pneumococcal pneumonia of rats and more effective than sulfanilamide in sepsis of mice. This serum had no therapeutic action in meningitis of rats caused by the same culture of Type III pneumococcus.

3 The rats which recovered from pneumococcal pneumonia showed a slight immunity which was least marked in those treated with sulfapyridine. No appreciable immunity was demonstrable in the rats which recovered from pneumococcal meningitis irrespective of their previous therapy.

4 Sulfapyridine treated mice which recovered from pneumococcal sepsis possessed less immunity than sulfanilamide-treated mice. In either group this immunity was not sufficiently great to save all mice reinjected with 100 fatal doses of homologous culture.

BIBLIOGRAPHY

- 1 Whithy, L. E. H., Chemotherapy of pneumococcal and other infections with 2 (p-aminobenzenesulfonamido) pyridine (M&B 693) *Lancet*, 1938, 1, 1210.
- 2 Evans, G. M., and Gaisford, W. R., Treatment of pneumonia with 2 (p-aminobenzenesulfonamido) pyridine (M&B 693) *Lancet*, 1938, 2, 14.
- 3 Cooper F. B., Gross P., and Lewis M., Chemotherapeutic evaluation of sulfanilamide and 2 (sulfanilamido) pyridine in Type II pneumococcal infections in mice and rats. *Proc. Soc. Exper. Biol. and Med.*, 1939 40, 37.
- 4 Hilles, C., and Schmidt, L. H., Sulfapyridine (2-p-aminobenzenesulfonamidopyridine) in experimental infections with Type XXII pneumococcus. *Proc. Soc. Exper. Biol. and Med.*, 1939 40, 73.
- 5 Long P. H., Bliss E. A., and Fernstone, W. H., The effects of sulfapyridine, sulfanilamide and related compounds in bacterial infections. *Pennsylvania M. J.*, 1939 42 483.
- 6 Cooper F. B., Gross, P., and Lewis, M., Chemotherapy of pneumococcal (Type II) meningitis in the rat. *Proc. Soc. Exper. Biol. and Med.* 1938, 38 835.
- 7 Gross, P., Cooper F. B., and Lewis, M., The chemotherapy of Type II pneumococcal meningitis. *Am. J. M. Sc.* 1939 197 609.
- 8 Gross, P., Cooper F. B., and Lewis, M., Therapeutics of experimental Type I pneumococcal meningitis in rats. *Am. J. M. Sc.* 1939 (In press)

- 9 Gross, P, and Cooper, F B, Efficacy of p-aminobenzenesulfonamide in experimental Type III pneumococcus pneumonia of rats *Proc. Soc. Exper Biol and Med.*, 1937, 36, 225
- 10 Gross, P, and Cooper, F B, p-aminobenzenesulfonamide and antipneumococcal serum therapy in Type I pneumococcal infections of rats *Proc. Soc. Exper Biol and Med.*, 1937, 36, 535
- 11 Cooper, F B, and Gross, P., para-aminobenzenesulfonamide therapy in experimental Type III pneumococcal pneumonia. *Proc. Soc. Exper Biol. and Med.*, 1937, 36, 678
- 12 Cooper, F B, and Gross, P, Sulfanilamide, anti-pneumococcus serum and vitamin C therapy in Type II pneumococcal pneumonia in rats *Proc. Soc. Exper Biol and Med.*, 1937, 36, 774
- 13 Rosenthal, S M, Studies in Chemotherapy II. Chemotherapy of experimental pneumococcus infections *Pub Health Rep*, 1937, 52, 48
- 14 Maclean, I H, Rogers, K. B, and Fleming, A, M&B 693 and pneumococci *Lancet*, 1939, 1, 562.
- 15 To be published.
- 16 Whitby, L E. H, The assessment of the efficiency of chemotherapeutic substances *Practitioner*, 1937, 139, 650
- 17 Whitby, L E H, Chemotherapy of bacterial infections *Lancet*, 1938, 2, 1095
- 18 Whitby, L. E. H, An experimental assessment of the therapeutic efficacy of amino compounds *Lancet*, 1937, 1, 1517
- 19 Cooper, F B, Gross, P, and Lewis, M, Chemotherapy of Types VII and III pneumococcal infections with sulphanilamide, 4,4'-di-(acetylamino)-diphenylsulphone and 4,4'-diaminobenzenesulphonanilide. *Am. J M Sc.*, 1938, 196, 343
- 20 Cooper, F B, Gross, P, and Mellon, R. R., Action of p-aminobenzenesulfonamide on Type III pneumococcus infections in mice. *Proc. Soc. Exper Biol and Med.*, 1937, 36, 148
- 21 Schmidt, L H, Use of sulfanilamide in the treatment of Type XIV pneumococcus infections in mice. *Proc. Soc. Exper Biol and Med.*, 1937, 37, 205
- 22 Long, P H, Bliss, E A., and Feinstone, W H, Mode of action, clinical use and toxic manifestations of sulfanilamide. *J A M A.*, 1939, 112, 115
- 23 Pneumonia Commission, Medical Society of the State of Pennsylvania, *Weekly Roster and Med. Digest*, 1938, 33, 647
- 24 Marshall, E. K., Jr, and Cutting, W C, Absorption and excretion of sulfanilamide in the mouse and rat. *Bull Johns Hopkins Hosp*, 1938, 63, 328
- 25 Feinstone, W H, Bliss, E A, Ott, E., and Long, P H, Observations concerning the toxicity, absorption and therapeutic effect of sulfanilamide and certain related organic sulphur-containing compounds in experimental infections in mice. *Bull Johns Hopkins Hosp*, 1938, 62, 565

ON VAGAL AND EXTRAVAGAL FACTORS IN CARDIAC SLOWING BY DIGITALIS IN PATIENTS WITH AURICULAR FIBRILLATION

By HARRY GOLD NATHANIEL T. KWIT HAROLD OTTO AND
THEODORE FOX

(From the Department of Pharmacology Cornell University Medical College the Hospital for
Joint Diseases and the Beth Israel Hospital New York City)

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The improvement of the circulation produced by digitalis in patients with auricular fibrillation is almost invariably associated with slowing of ventricular rate. Whether the improvement is the cause of the slowing or the result of it, or in part a cause and in part a result of the slowing are matters concerning which opinion is divided. The extent to which the slowing causes the improvement could be established if the slowing could be prevented. It appeared that this problem might be attacked by the use of repeated doses of atropine to prevent the cardiac slowing over a period of several days after a massive dose of digitalis. The influence of digitalis on the heart failure as measured by changes in body weight fluid loss, and subjective symptoms in these cases might then be compared with its well known effect in patients in whom the rate is permitted to become slow.

The effect of atropine on the heart rate of digitalized patients with auricular fibrillation has been the subject of several reports in the literature, and while views were not entirely in agreement there seemed to be sufficient accord to indicate that with adequate doses of atropine little difficulty would be encountered in maintaining a rapid heart rate after large doses of digitalis in a considerable proportion of the patients.

A study was planned to pursue this matter, but the original objective ceased to appear promising after several results were obtained, for they showed what was confirmed by subsequent observations, that the experiment could not be carried out, that atropine, even in doses causing severe systemic symptoms, could not prevent the pronounced slowing of the ventricle after very large doses of digitalis. The inference we drew from the literature, therefore, proved to be incorrect, and a reexamination of the literature disclosed the fact that the discordant observations and conflicting conclusions concerning the role of the

vagus could not be harmonized by existing data alone.

LITERATURE

While there are numerous reports on the effect of atropine on the ventricular rate which has been slowed by digitalis, the studies of Cushny Marris and Silberberg (1), and Lewis, Drury Wedd and Iliescu (2) present the more detailed analyses of the subject. Cushny *et al.* (1) concluded that "in auricular fibrillation, digitalis and its allies slow the heart from some direct action on the heart and not from stimulation of the inhibitory mechanism, for atropine does not restore the original rate of the released heart." About 10 years later Lewis *et al.* (2) on the other hand, concluded that the ventricular slowing caused by digitalis is in part due to a direct action on the ventricle and in part to vagal stimulation, and that the different proportions of these actions in different cases were chiefly matters of individual variation, both actions being exerted "in different proportions from case to case." In the 10 digitalized patients of Cushny *et al.* the average ventricular rate after atropine was only 79 a minute, whereas in the 8 patients of Lewis *et al.* it was 137 a minute. Lewis *et al.* attributed this difference to the inadequate doses of atropine used by Cushny *et al.* for Lewis found that after digitalis it took more atropine completely to block the vagus than before digitalis, and that a dose of $\frac{3}{160}$ grain subcutaneously (doses used by Cushny *et al.*) was not sufficient. In his review of these earlier observations, Cushny (3) stated, "it seems possible that the relative importance of the two factors may vary with different doses of the drug." He suggested that the dissimilar results were not due to differences in the doses of atropine but of digitalis. He stated that the evidence of more intense digitalization in his own cases was a greater fall in the pulse rate. The records however do not support this statement, for the average pulse rates in the 2 series of cases were practically identical. Cushny *et al.*, before digitalis 103 per minute, after digitalis 65. Lewis *et al.*, before digitalis 106, after digitalis 65. The different results obtained in the two series of cases remained therefore unexplained. Cushny suggested other factors which might determine whether the rate was slowed chiefly by one or the other mechanism. Individual peculiarities, the condition of the heart, and the length of time that the heart had been under the

"the longer the drug acts the less the role played by the vagus, and the greater that of direct impairment of the A-V fibers"

In his monograph, Robinson (4) called attention to the prevailing uncertainty as to whether the action of digitalis on conduction "should be regarded mainly as an effect of vagus stimulation or as an effect produced by the direct action of the drug on the heart." More recently (5) Elsie Porter reported some new experiments on subjects with auricular fibrillation from which she took the position that the cardiac slowing is entirely vagal, and that "if and when a larger dose of atropin can be safely given intravenously, the whole digitalis effect will be found to fall upon the vagus"

In view of the unsettled state of the matter, our experiments were extended to explore further the effect of maximum doses of atropine on the heart rate in auricular fibrillation after various doses of digitalis in one and the same individual, in different individuals, and under different conditions. The results of these are presented in the present report.

METHOD

Patients The experiments were performed on 9 subjects with organic heart disease. In 8 of these there was an advanced degree of heart failure with congestion, and in 1 there were no signs or symptoms of heart failure. All had a persistent auricular fibrillation. The characteristics of the group are summarized in Table I. The patients were admitted to the hospital and kept in bed. In addition to the routine laboratory tests, includ-

TABLE I

Description of patients with auricular fibrillation used in this study

Case	Age	Sex	Weight	Diagnosis
	<i>years</i>		<i>pounds</i>	
1	65	Male	182	Hypertension enlarged heart
2	42	Male	183	Unknown etiology enlarged heart
3	44	Female	93	Unknown etiology mitral stenosis and insufficiency enlarged heart
4	55	Male	134	Hypertension and arteriosclerosis, enlarged heart, syphilis
5	44	Male	155	Unknown etiology mitral stenosis and insufficiency enlarged heart
6	35	Female	130	Rheumatic fever mitral stenosis and insufficiency enlarged heart
7	56	Male	130	Rheumatic fever mitral stenosis and insufficiency aortic insufficiency enlarged heart
8	29	Male	131	Rheumatic fever mitral stenosis and insufficiency aortic stenosis and insufficiency enlarged heart
9*	40	Female	135	Rheumatic fever mitral stenosis and insufficiency enlarged heart, developed auricular fibrillation after hemorrhoidectomy while in the hospital had had thyroidectomy 18 years ago for exophthalmic goiter but now has a normal metabolic rate

ing electrocardiograms and the daily clinical examinations, their fluid intake and output, and ventricular and pulse rates were charted. They were weighed on admission and again usually every third day. The fluid intake was fixed at approximately a liter a day. The control period was continued until a fairly fixed level was reached for ventricular rate, body weight, and water balance. The control period lasted up to 16 days, but was terminated as early as 3 days after admission in 3 cases in which the heart failure appeared to be growing rapidly worse. During the control period digitalis and diuretics were withheld.

Digitalis The drug was administered in the form of compressed tablets of the powdered leaf standardized by the cat method. All but Case 9 received digitalis with a potency of 97 mgm. per cat unit. A dose of 0.14 to 0.2 cat unit per pound of body weight (24 to 54 grains) was given at one time in 6 cases, in 4 of these, additional doses were given subsequently. Two cases received smaller doses at the start, but a total of 0.14 and 0.46 cat unit per pound in 2 and 13 days respectively. These doses were sufficient to induce toxic symptoms in 4 of the 9 patients (Table II). In calculating the dose, no allowance was made for the amount of edema fluid. Case 9 received a specimen of digitalis with a cat unit potency of 85 mgm.

Atropine Enough atropine was given to abolish completely the vagal control of the ventricular rate. The dose which appeared sufficient to accomplish this was $\frac{1}{30}$ gram (2.16 mgm.) of atropine sulfate by intravenous injection, as shown by the fact that the release of the heart rate produced by this dose was in no instance increased by more of the drug (tested in 6 experiments). In all, 36 intravenous doses of atropine sulfate were administered in a concentration of 0.2 per cent in physiological salt solution. These were given in single doses of $\frac{1}{60}$ gram or 1.08 mgm (3 doses), $\frac{1}{30}$ gram or 2.16 mgm (25 doses), $\frac{1}{25}$ gram or 2.6 mgm (5 doses), $\frac{1}{20}$ gram or 3.2 mgm (2 doses), and $\frac{1}{15}$ gram or 4.3 mgm (1 dose). In one case a total of $\frac{1}{11}$ gram (6 mgm) was given in 2 hours and in another a total of $\frac{1}{5}$ gram (13 mgm.) was given in 24 hours. As already indicated, the maximum effect on the rate resulted from $\frac{1}{30}$ gram, and larger doses served merely to intensify the toxic symptoms. These doses are substantially similar to those found necessary to obtain maximum ventricular release in experiments by Lewis, Drury, Wedd, and Ilescu (2).

The ventricular rate was counted with the stethoscope at the apex, a count of a half minute being taken at intervals of 1 to 2 minutes at first, and then at longer intervals. In one case the rate was counted on electrocardiograms taken at similar intervals. The maximum acceleration of the rate was in evidence within a minute or two. Partial recovery, however, occurred fairly rapidly, as much as a half of the acceleration might disappear within about 15 minutes. The complete return of the rate to the pre-atropine level after the dose of $\frac{1}{30}$ gram was more gradual, sometimes not quite complete even after about 5 or 6 hours. Some delay in the return may be due to restlessness induced by the atropine. It

* All had advanced congestive heart failure, except this patient who never had signs or symptoms of failure

TABLE II

Summarising effect of atropine on ventricular rate after different doses* of digitalis

† Case	Ventricular rate before digitalis	Total digitalis dose	Toxic effect of digitalis	Interval between beginning of digitalisation and atropine	Ventricular rate		Per cent of the digitalis slowing abolished by atropine
					Before atropine	After atropine	
	per min	grains			per min	per min	per cent
A † PREDOMINANTLY VAGAL ACTION (SMALLER DOSES)							
1	148	54	None	2 hours	133	152	100 (127)‡
2	145	42	None	2 hours	114	164	100 (161)
		42	None	4 days	71	161	100 (132)
3	128	24	None	10 hours	81	141	100 (128)
		24	None	22 hours	61	140	100 (117)
4	118	30	None	8 hours	93	138	100 (140)
		42(a)	None	19 hours	76	105	70 (70)
8	85	27(b)	None	4 days	58	85	97 (97)
9	134	60(c)	None	8 days	90	133	98 (98)
B PREDOMINANTLY EXTRAVAGAL ACTION (LARGER DOSES)							
1	148	54	Vomited(d)	23 hours	75	92	23
2	145	78(e)	None	14 days	56	70	16
3	128	117(f)	Vomited	10 days	75	100	47
		117(f)		10 days	73	90	28
		117(f)		6 hours	43	88	32
		117(f)		11 days	43	88	32
4	118	75(e)	Coughing	12 days	60	90	32
5	134	35	Vomited(h)	8 hours	64	89	35
		56(f)	None	8 days	64	88	34
6	115	27	None	5 hours	84	105	68
		27	None	2 days	86	99	43
7	132	93(f)	None	18 days	52	68	20
9	134	87(c)	None	13 days	80	97	32

* Unless otherwise stated the entire dose was given at one time.

† Cases 1, 2, 3, 4 and 9 were tested after both smaller and larger doses

‡ Borderline cases could not be satisfactorily classified

§ Figures in this column indicate not only release of digitalis slowing but acceleration above the rate in the control prior to digitalis

(a) 12 grains more on Day 1 (In all cases, the days are reckoned from the first day on which digitalis is given.)

(b) 15 grains on Day 1 and 12 grains on Day 2.

(c) See Figure 6

(d) 9 hours after digitalis.

(e) 12 grains on Days 6, 7 and 8.

(f) 12 to 18 grains daily on Days 3 to 9

(g) 12 grains on Days 5 and 6, 6 grains on Day 7, and 3 grains on Day 10

(h) 11 hours after digitalis.

(i) 3 grains daily on Days 2 to 8

(j) 21 grains on Day 1, 12 grains on Days 2 to 5 and 3 grains on Days 6 to 13

may be inferred that the fairly rapid recovery of the slow heart rate after a dose of atropine is due, at least in part, to mechanisms other than elimination of the drug, since additional doses, even larger ones usually

failed to raise the ventricle to the maximum rate prevailing after the first dose, when the second was given during what appeared to be considerable recovery from the first. Figure 1 illustrates the types of atropine curves. Lewis *et al* (2) also observed the rapid recovery and attributed it in part to reflex stimulation of the vagus center (increased afferent impulses playing on the vagal center) as the result of the sudden cardiac acceleration. The possibility of sympathetic depression (synapse of the ganglion) or some direct muscular depression by atropine cannot be excluded. In connection with another study (6) we observed brief sinus slowing and prolonged P-R intervals after a large dose of atropine in digitalized animals. Similar effects under special conditions were reported by Lewis *et al* (2). Whether such effects play any part in the return to the slow rate after atropine cannot be stated.

RESULTS

Atropine cannot prevent cardiac slowing Figure 2¹ shows the course of events in the endeavor to secure full digitalis effect without ventricular slowing. In Case 2, the first dose of atropine ($\frac{1}{10}$ grain) was given 1 hour and 46 minutes after the oral dose of 42 grains of digitalis. A second similar dose of atropine was given 1 hour and 27 minutes after the first and the third dose of $\frac{1}{10}$ grain was given 1 hour and 34 minutes after the second, in all $\frac{3}{10}$ grain in a period of 3 hours and 1 minute. These doses caused atropine poisoning with stupor, restlessness, muttering, dryness and dilated pupils, symptoms which became more intense as the doses were repeated. As may be seen, however, the release of the ventricle was only temporary, was diminished with the repetition of the dose, and the release was almost negligible after the last dose, indicating that the vagus was in all probability completely blocked. Nevertheless, the ventricular rate continued to fall as the absorption of digitalis progressed, and about 12 hours after the administration of the drug the rate had fallen from 145 to 85 while the patient was under the influence of sufficient atropine to cause severe toxic effects. In Case 1, the phenomena were essentially the same with even larger doses of atropine.

These results show, therefore, that even when

¹ In order to simplify inspection of these curves some of the points which come very close together have been omitted without significantly influencing the course of the curves. This sometimes had the effect of smoothing out minor irregularities in the curves.

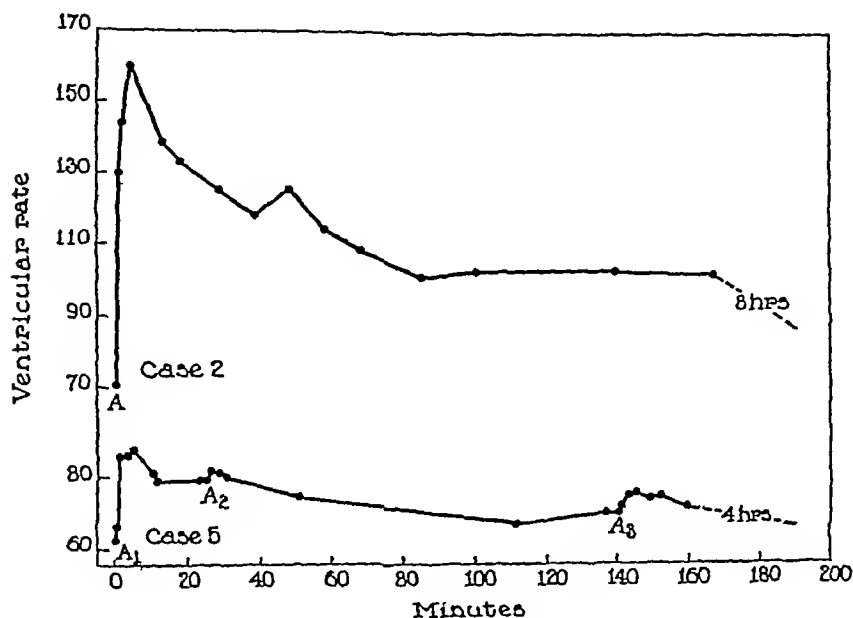


FIG 1 TYPES OF ATROPINE EFFECTS

Case 2 (A) represents $\frac{1}{60}$ grain atropine sulfate given intravenously after marked slowing by a full dose of digitalis

Case 5 Each (A) represents $\frac{1}{60}$ grain atropine sulfate given intravenously after marked slowing by a full dose of digitalis

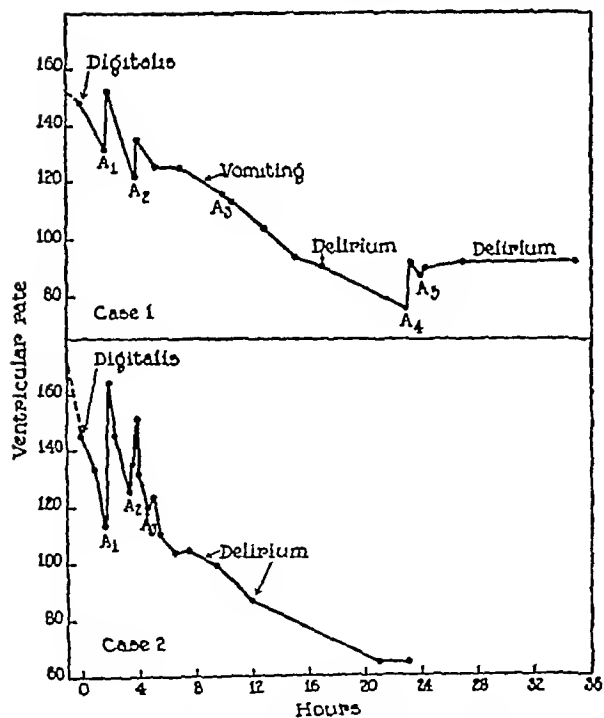


FIG 2. ATROPINE CANNOT PREVENT SLOWING BY DIGITALIS

Case 1 Dose of digitalis, 54 grains A1, A2, A3, A4, A5 (atropine sulfate, $\frac{1}{25}$ grain each)

Case 2 Dose of digitalis, 42 grains A1, A2 (atropine sulfate, $\frac{1}{30}$ grain each) A3 (atropine sulfate, $\frac{1}{60}$ grain)

the vagi are blocked by atropine, marked ventricular slowing is induced by sufficiently large doses of digitalis. This conclusion was subsequently confirmed by the results obtained in the remaining cases, as may be seen in Table II.

Vagal and extravagal mechanisms shown by the atropine test At the extremes two types of effect may be seen after large doses of digitalis. These are illustrated in Figure 3. These patients both had advanced congestive heart failure with a rapid ventricular rate (148 and 128 a minute). Each received a large dose of digitalis (0.2 and 0.15 cat unit per pound of body weight), and in each, nearly 24 hours later, the ventricular rate had declined to low levels (75 and 61 a minute). Despite these essentially similar conditions, the blocking of the vagus by atropine had little effect in one and a very marked effect in the other. Of these, in one, only 23 per cent of the digitalis slowing was removed, while in the other 100 per cent of the digitalis slowing was abolished, the ventricular rate rising higher than the control rate before the digitalis. In Case 3, therefore, the action of the digitalis was predominantly vagal, and in Case 1, predominantly extravagal (direct action on conduction²).

² Luten (7) refers the extravagal factor, in part at least, to reduced excitability of the ventricle.

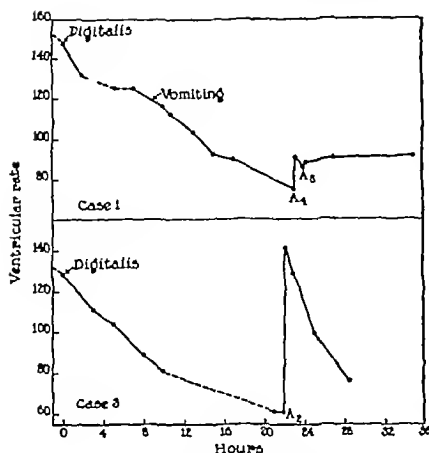


FIG. 3. VAGAL AND EXTRAVAGAL MECHANISM OF SLOWING

Case 1 Dose of digitalis, 54 grains. A4 A5 (atropine sulfate, $\frac{3}{16}$ grain each) In broken lines changes in rate due to previous doses of atropine are omitted.

Case 3 Dose of digitalis, 24 grains. A2 (atropine sulfate, $\frac{1}{16}$ grain) Broken line of same significance as in Case 1

Factors which determine the mechanism The next question was What factors determine whether one or the other mechanism would control the ventricular rate after digitalis—individual peculiarity, the degree of heart failure or of functional improvement or the dosage of the drug?

Figure 4 shows that it is not a matter of individual peculiarity, for in the same individual the slow heart rate during digitalis action may be at one time under vagal control and at another time under extravagal control. For example, in Case 2, the control is extravagal 10 hours after 42 grains of digitalis, it is the vagal type (100 per cent release) after 4 days but it again returns to the extravagal control (only 16 per cent release) on the fourteenth day. Similarly in Case 3 the heart rate is slow by a vagal mechanism on the first and second day after the digitalis, but by a predominantly extravagal mechanism on the tenth and eleventh day. In Table II are summarized the observations in all the experiments indicating that one and the same individual may show both types of control.

Figure 5 shows that the mechanism which will control the slow heart rate after digitalis is not directly related to the degree of heart failure. In Case 5 the same mechanism, the extravagal one, which controlled the ventricular rate during the period in which the symptoms of failure had almost completely subsided (loss of 12 pounds of edema fluid) on the eighth day after beginning digitalis was already in control 8 hours after the first massive dose of 35 grains when relatively little clinical improvement was in evidence. In Case 2 the extravagal mechanism predominated in the slowing about 8 hours after 42 grains of digitalis, as shown by the fact that even though the vagi were blocked by atropine the rate did not exceed 104 (reduced from 145). During this period very little clinical improvement was in evidence. By the fourth day marked clinical improvement had taken place, the patient having lost 10 pounds of edema fluid. At this time the control had shifted to the vagal mechanism (rate after atropine release, 161).

Figure 6 shows that the two mechanisms may be seen at different times in a patient without signs or symptoms of heart failure.

The foregoing results show that the vagal and extravagal types of control of the ventricular rate may alternate in the course of digitalization in a manner that seems in no way related to the state of the myocardial function as revealed by the clinical signs and symptoms.

In Table II the effect of the size of the dose on the mechanism is shown. The dose of digitalis appears to be the only factor consistently related to the mechanism. It is not safe to compare one case with another with respect to the amounts of drug because of differences in tolerance, but the course of events in individual cases shows that when the dose is increased the atropine test reveals a shift from the vagal to the extravagal control of the ventricular rate and *vice versa* when the dose acting upon the heart is reduced. For example, in Case 1, a predominantly vagal type (Case A1 100 per cent release) 2 hours after the dose of digitalis became a predominantly extravagal type (Case B1 23 per cent release) after more digitalis had been absorbed in 23 hours following its administration. In Case 2 there is the extravagal type of control 8 hours after the large dose of digitalis (see also, F. 5).

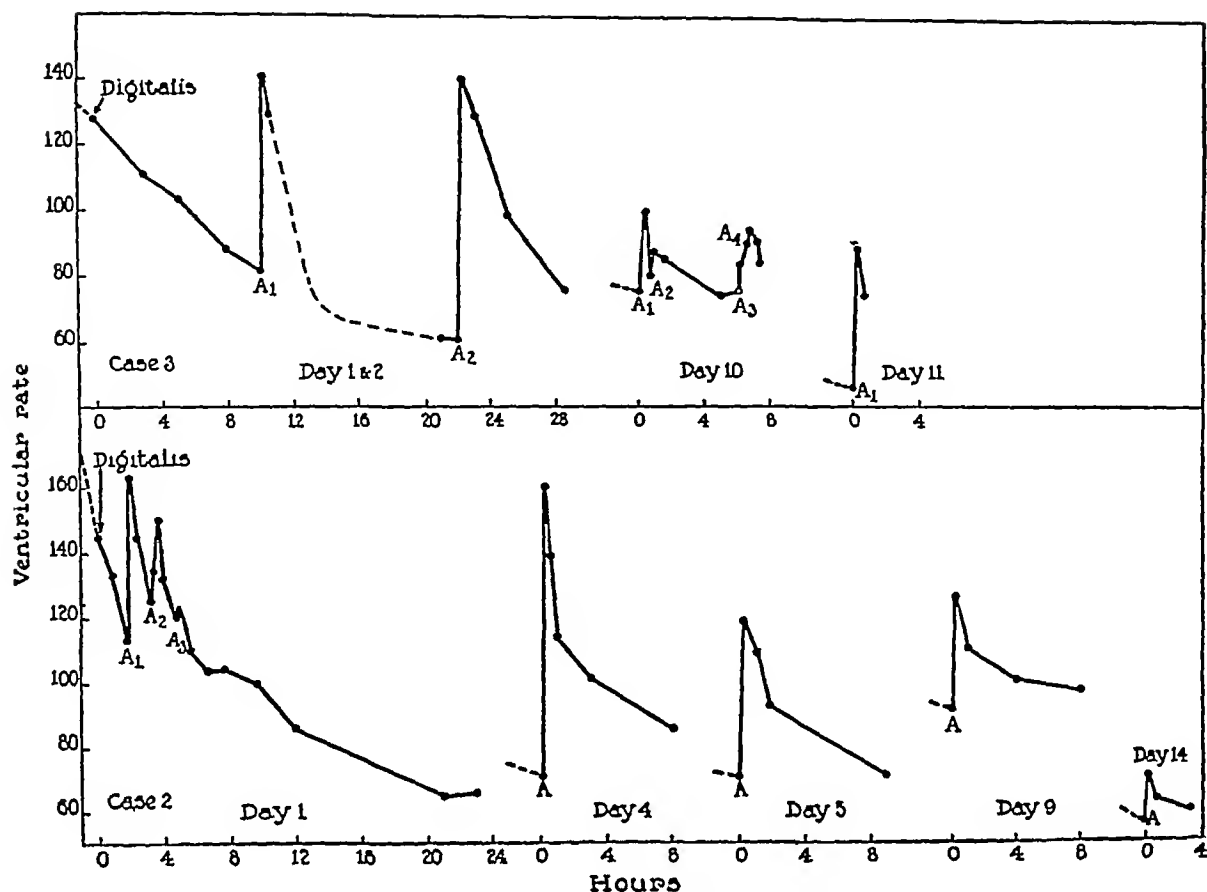


FIG 4 EITHER VAGAL OR EXTRAVAGAL CONTROL OF THE VENTRICULAR RATE MAY PREVAIL IN THE SAME INDIVIDUAL

Case 3 Dose of digitalis, 24 grains on Day 1, 93 grains more during Days 3 to 9 Each (A) represents $\frac{1}{80}$ grain atropine sulfate. Broken line (no observations during the night)

Case 2 Dose of digitalis, 42 grains on Day 1, 36 grains more during Days 6 to 8 Each (A) represents $\frac{1}{80}$ grain atropine sulfate, except A3, and A on Day 5 ($\frac{1}{60}$ grain each)

vagal type (100 per cent release) in 4 days, during which time some of the drug was eliminated, but a return to extravagal control (only 16 per cent release) on the fourteenth day and after more digitalis, a total of 78 grains

The results in Table II also show that atropine invariably causes some acceleration of the ventricular rate after it has been slowed by digitalis in patients with auricular fibrillation. This applies not only to small doses of digitalis but to maximum doses, those causing nausea and vomiting. The extent to which the most effective dose of atropine will raise the ventricular rate depends upon the degree of the digitalization. After the smaller doses of digitalis the slowing can be entirely abolished by atropine, but after the larger ones the slowing can be only partially abolished

by atropine. During the full action of the maximum doses it is not possible to accelerate the ventricular rate appreciably above 100 a minute. In these cases the maximum rate after complete vagal release by atropine is usually considerably below 100 a minute.

COMMENT

We are now in a better position to comprehend the conflict in the literature regarding the rôle of the vagus in digitalis slowing. In Table III the essentials of three studies have been summarized and compared with the results obtained in our own. An inspection of this table shows that the results in all are qualitatively identical, but quantitatively different and these differences appear clearly to be related to the differences in

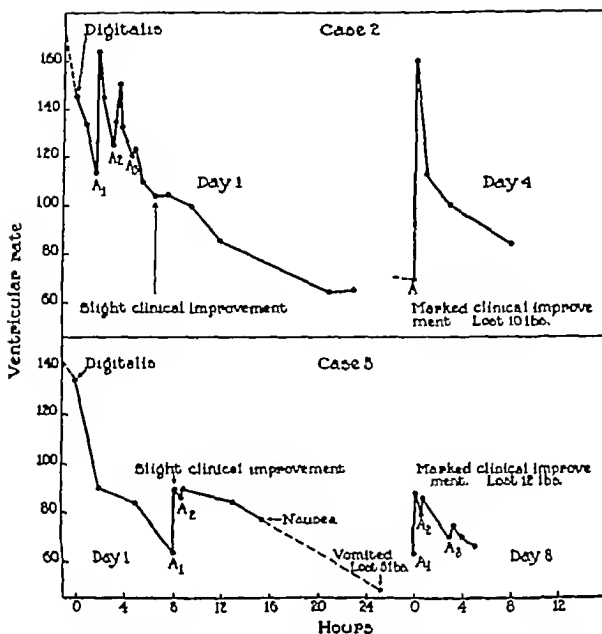


FIG. 5 IMPROVEMENT OF HEART FAILURE DOES NOT DETERMINE THE MECHANISM OF THE SLOWING

Case 2. See legend of Figure 4

Case 5. Dose of digitalis, 35 grains on Day 1 and 21 grains more during Days 2 to 8. Each (A) represents $\frac{1}{60}$ grain atropine sulfate.

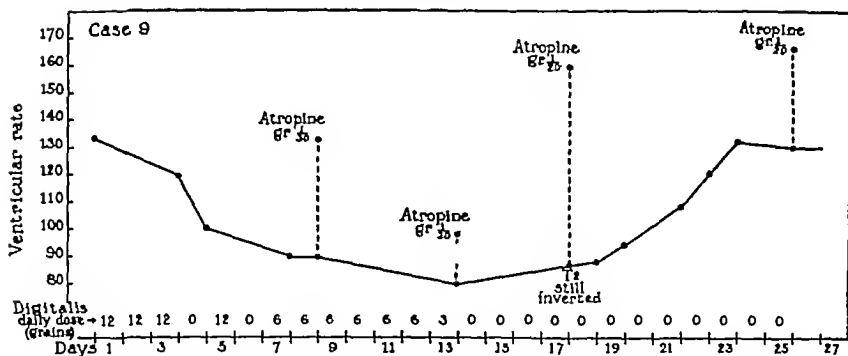


FIG. 6. VAGAL AND EXTRAVAGAL MECHANISM OF SLOWING IN A PATIENT WITHOUT SIGNS OR SYMPTOMS OF HEART FAILURE

TABLE III

Effect of atropine on ventricular rate after digitalis in 4 studies with patients having auricular fibrillation

Author	Number of cases	Average ventricular rate without digitalis	Dose of digitalis	Toxic effects	Average ventricular rate after digitalis and before atropine	Dose of atropine sulfate	Average ventricular rate after atropine	Per cent of digitalis slowing abolished by atropine
Cushny, Marris, and Silberberg, 1912 (1)	11 experiments in 10 cases	<i>per minute</i> 103 (75-125)	0.8 dram of tincture daily for 13 days (0.3 to 1 dram daily for from 5 to 32 days)	Nausea, vomiting or coupling in 9 cases	<i>per minute</i> 65 (52-77)	<i>grains</i> Usually 1/50 subcutaneously	<i>per minute</i> 79 (57-93)	37
Lewis, Drury, Wedd, and Iliescu, 1922 (2)	8 experiments in 7 cases	106 (80-130)	0.8 dram of tincture daily for 10 days (0.7 to 1.1 dram daily for from 7 to 13 days)	Not stated	65 (52-75)	Usually 1/33 to 1/20 intravenously	137 (102-205)	100 (175)*
Porter, 1933 (5)	21	128 (72-180)	0.12 cc of tincture per lb. of body weight (0.1 cc to 0.15 cc) administered in 1 dose 8 hours before atropine	Vomiting in 1 case	101 (72-156)	1/25 intravenously	Not stated	100+ in 1/3 of the cases, less than 100 in 2/3 of the cases
Present study	9 experiments in 6 cases	126 (86-148)	About 0.15 cat unit per lb. of body weight (see Table II details)	None	86 (58-133)	Usually 1/30 or more intravenously	134	100 (124)*
	12 experiments in 8 cases	132 (115-148)	0.2 to 0.5 cat unit per lb. of body weight (see Table II details)	Coupling or vomiting in 4 cases	68 (52-86)	Usually 1/30 or more intravenously	89 (68-105)	34

* These figures above 100 indicate not only the release of digitalis slowing but the extent of the acceleration above the pre-digitalis rate

the amount of digitalis given. In one and the same patient we obtained the results of Cushny *et al* (1) when the doses were large, and the results of Lewis *et al* (2) and of Porter (5) when the doses were smaller. It is not possible to compare the doses of digitalis accurately because we have no data concerning their relative potencies, but the effects indicate that the intensity of digitalization was not the same in the three studies. Cushny *et al* drew their conclusions from cases of advanced digitalization as seen from the high incidence of toxic effects³. The cases of Lewis *et al* were evidently less intensively digitalized, although no statement is made regarding toxic

³ Although their inferences are also invalid on the grounds of inadequate doses of atropine as we have already mentioned, in the light of our experiments it is improbable that even larger doses of atropine would have yielded materially different results.

symptoms. The cases of Elsie Porter were, on the other hand, in the lightest degree of digitalization as indicated chiefly by the relatively high average ventricular rate at the time that the atropine test was made. It may be noted, also, that the ventricular slowing in each of three groups of experiments was almost identical, rate 65 (Cushny), 65 (Lewis), and 68 (present study). Slow rate, therefore, was no indication of the mechanism by which the slowing was caused, for in one group with this slow rate atropine abolished the slowing completely and in the other the effect of atropine was relatively slight. This matter will be considered in another communication.

SUMMARY AND CONCLUSIONS

1 There is no general agreement regarding the rôle of the vagus in the ventricular slowing by

digitalis, some maintaining that the drug acts mainly on conduction directly, and others, that its action is mediated chiefly or wholly through the vagus

2 In the present investigation on patients with auricular fibrillation we found, as others have, that paralytic doses of atropine always cause some acceleration of the ventricle which has been slowed by digitalis, and that this effect varies from slight acceleration to complete abolition of the slowing

3 We have observed however, that if the doses of digitalis are large enough, atropine cannot prevent digitalis from producing marked slowing of a rapid ventricle (to 100 a minute or slower)

4 In digitalized patients with auricular fibrillation, the ventricle is maintained at a slow rate usually by the summation of two factors one, a vagal factor (abolished by atropine) and two an extravagal factor (not abolished by atropine)

5 In the average case, the vagal factor predominates in the slowing of the ventricle after moderate doses of digitalis while the extravagal factor predominates after large doses

6 Which of the two factors (vagal or extravagal) will dominate in the control of the slowed heart rate depends therefore upon the degree of digitalization. Contrary to statements found in the literature, our results show that it is not a

matter of individual peculiarity, the degree of heart failure or the length of time the heart has been under the influence of digitalis

7 The discordant views in the literature regarding the role of the vagus, and the factors which alter its role, in slowing of the ventricle in auricular fibrillation arise from the failure to make adequate observations on the effect of maximum doses of atropine after different doses of digitalis in one and the same subject

BIBLIOGRAPHY

1. Cushny A. R., Marris, H. F., and Süßberg M. D., The action of digitalis in therapeutics. *Heart* 1912, 4, 33
2. Lewis, T., Drury A. N., Wedd A. M., and Iliescu, C. C., Observations upon the action of certain drugs upon fibrillation of the auricles. *Heart*, 1922, 9 207
3. Cushny A. R., *The Action and Uses in Medicine of Digitalis and its Allies*. Longmans Green and Co., London, 1925
4. Robinson, G. C., *The Therapeutic Use of Digitalis* Williams and Wilkins Co., Baltimore, 1923
5. Porter E., The therapeutic use of drugs of the digitalis group. *Quart. J. Med.*, n. s., 1933 2, 33
6. Gold, H., Lieberman, A., and Gelfand, B., Mechanism of production of subauricular beats by digitalis bodies. *Arch. Int. Med.* 1931 48, 262.
7. Luten, D. *The Clinical Use of Digitalis*. Charles C. Thomas Baltimore, 1936

MECHANISM OF THE ARTERIAL HYPERTENSION INDUCED BY PAREDROLINOL (α -N-DIMETHYL-p- HYDROXYPHENETHYLAMINE)

By EUGENE A. STEAD, JR., AND PAUL KUNKEL

(From the Thorndike Memorial Laboratory Second and Fourth Medical Services (Harvard)
Boston City Hospital and the Department of Medicine Harvard Medical School Boston)

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In arterial hypertension the cardiac output the metabolic rate and the usual blood chemical constituents are essentially normal. Experimental hypertension in man, however is usually attended by alterations in some of these measurements. Epinephrine produces hypertension in normal subjects but at the same time it usually increases the heart rate and cardiac output. It elevates the basal metabolism, the blood flow in the muscles the blood sugar and the blood lactic acid and also causes great vasoconstriction in the skin vessels. This hypertension is therefore in no way comparable to that observed in disease. The study to be reported indicates that in normal subjects paredrolinol sulphate¹ (α -N-dimethyl p-hydroxyphenethylamine sulphate) produces a type of hypertension which has many features in common with clinical hypertension. For this reason the circulatory adjustments following the administration of this drug are of particular interest.

The pharmacology of paredrolinol, a sympathomimetic drug closely allied in structure to ephedrine, has been studied extensively in animals. Rein (1, 2), in an investigation of the action of paredrolinol on dogs anesthetized with morphine and pernocton found that both the arterial pressure and the minute volume output of the heart were elevated. The drug acted first on the venous side of the circulation and produced a greater venous return to the heart by increasing the venous tone and probably by emptying out the liver. This was followed in a few seconds by a slow increase in arterial tone and shortly thereafter by a powerful discharge of blood from the abdominal organs particularly the spleen. Rein claimed that this intense discharge of blood from

the abdominal venous reservoirs indicated that the main cause for the rise in arterial pressure was an increase in venous return to the heart, rather than an increase in peripheral resistance. He demonstrated that the blood flow in the extremities and in the abdominal viscera was never greatly decreased and at times was increased. Since the drug did not cause blanching when introduced into the human skin he concluded that the capillaries were not constricted and that the arterial tone was increased in the larger vessels.

Heymans and Bayless (3) on the basis of experiments conducted on anesthetized dogs, concluded that the vascular effect of the drug was characterized by slight peripheral vasoconstriction and pronounced splanchnic vasoconstriction. When the physiological reflexes for the proprioceptive regulation of blood pressure were depressed by means of barbiturates they were not restored by paredrolinol even though the blood pressure was raised. Lindner (4) working with isolated cats hearts demonstrated that paredrolinol in concentrations of 1 to 1,000,000 increased the frequency and strength of the heart beat.

Numerous observations have been made on the effect of paredrolinol in human subjects. The drug was found to be active by oral, rectal, subcutaneous, intramuscular and intravenous routes (5, 6, 7). It caused a rise in blood pressure, a fall in heart rate, and palpitation (5, 7). There were no other symptoms unless the blood pressure rose excessively in which event a sensation of severe pressure in the head and of precordial discomfort was experienced (5, 7, 8, 9). The venous pressure was increased by about 20 mm of water (7), and one observer reported that the rise in venous pressure occurred after the rise in arterial pressure (9). The skin of the subjects showed no change in color (5). Nodal rhythm and ventricular extrasystoles occurred in certain instances (10). There was no change in the level of the

¹ The paredrolinol sulphate used in this study was obtained through the courtesy of the Smith, Kline, and French Laboratories. This drug has been reported in the German literature under the trade names "veritol" and "H 75" (Knoll).

blood sugar (5) A transient rise in oxygen consumption has been reported (7) following the intravenous administration of paredrinol It was suggested that this increase in oxygen consumption was the result of a heightened flow from the large veins and venous reservoirs, and that the mobilization of blood from venous reservoirs, which Rein had demonstrated in the dog, also occurred in man The cardiac output was increased (11) when determined by the method of Broemser and Ranke, but in man this method was found to be unreliable in this laboratory Several authors (8, 9, 11) believe that the rise in blood pressure was produced both by an increased venous return to the heart and by an increase in the peripheral resistance The previous injection of atropine (12) prevented slowing of the heart and caused the blood pressure to rise to a higher level than that observed after the administration of paredrinol alone Paredrinol is the N-methyl derivative of paredrine (β -4-hydroxyphenylisopropylamine) The effect of paredrine on the heart rate, blood pressure, and skin temperature has been reported by Abbott and Henry (13)

METHOD

This study of the action of paredrinol was carried out on subjects with normal cardiovascular systems The arterial blood pressure was determined in the upper arm by the auscultatory method, using a mercury manometer The heart rate was counted by arterial palpation The blood flow in the hand, foot, forearm, and calf was measured by the plethysmographic methods previously described (14, 15, 16) When measurements were made on the forearm and calf, the circulation to the hand and foot distal to them was occluded by pressure cuffs below the plethysmographs (16, 17) The venous tone in the hand was measured by the method of Capps (18), and the venous pressure by the direct method of Moritz and Tabora (19) The cardiac output was measured by the acetylene method (20), and the basal metabolism by oxygen consumption The histamine method was used for determining the circulation time (21) Skin temperatures were measured by a thermocouple The paredrinol was injected intramuscularly after the subjects had rested quietly in the horizontal position for at least 30 minutes The effects of the upright position on the circulation were determined by tilting the table on which the subjects rested to an angle of from 30 to 75 degrees above the horizontal

RESULTS

Arterial pressure, venous pressure, and heart rate In 10 normal subjects the intramuscular

injection of 25 mgm of paredrinol raised the arterial blood pressure from an average of 120 mm Hg systolic and 76 mm diastolic to 173 mm systolic and 92 mm diastolic (Figure 1) The height to which the arterial pressure rose varied greatly in different subjects The minimum rise in arterial pressure was from 120 mm systolic and 70 mm diastolic to 148 mm systolic and 72 mm diastolic, the greatest was from 130 mm systolic and 78 mm diastolic to 200 mm systolic and 104 mm diastolic There was also great variation in the results obtained in the same subject, the arterial pressure on one day rising from 114 mm systolic and 80 mm diastolic to 190 mm systolic and 90 mm diastolic, while a few days later the same amount of paredrinol caused a rise in pressure to only 148 mm systolic and 72 mm diastolic The arterial blood pressure following intramuscular administration of 25 mgm of paredrinol began to rise in from 3 to 9 minutes (average 5 minutes) The maximum height was reached in from 8 to 20 minutes (average 16 minutes) The blood pressure returned to the resting level in from 40 to 70 minutes (average 57 minutes) The heart rate in these 10 subjects dropped from an average of 72 to an average of 63 beats per minute Three cases showed no significant change in heart rate The maximal change was from 81 to 54 beats per minute

In 3 normal subjects, from 35 to 40 mgm of the drug were given intramuscularly, the arterial blood pressure rose from normal to 180, 186, and 214 mm systolic, and to 80, 108, and 110 mm diastolic, respectively In 1 subject the arterial blood pressure was maintained above 190 mm systolic and 110 mm diastolic for 30 minutes by the repeated administration of smaller doses of paredrinol In a second subject an arterial pressure of from 180 to 196 mm systolic and 86 mm diastolic was maintained for 30 minutes

In 3 normal subjects the venous pressure was measured by the direct method and was found to increase by from 30 to 40 mm of water above the resting level The arterial and venous pressures began to rise at approximately the same time, but as neither determination was absolutely continuous it was not possible to say which was the first to increase In one subject, who had received 3 mgm of atropine subcutaneously before the intramuscular injection of 25 mgm of paredrinol, the

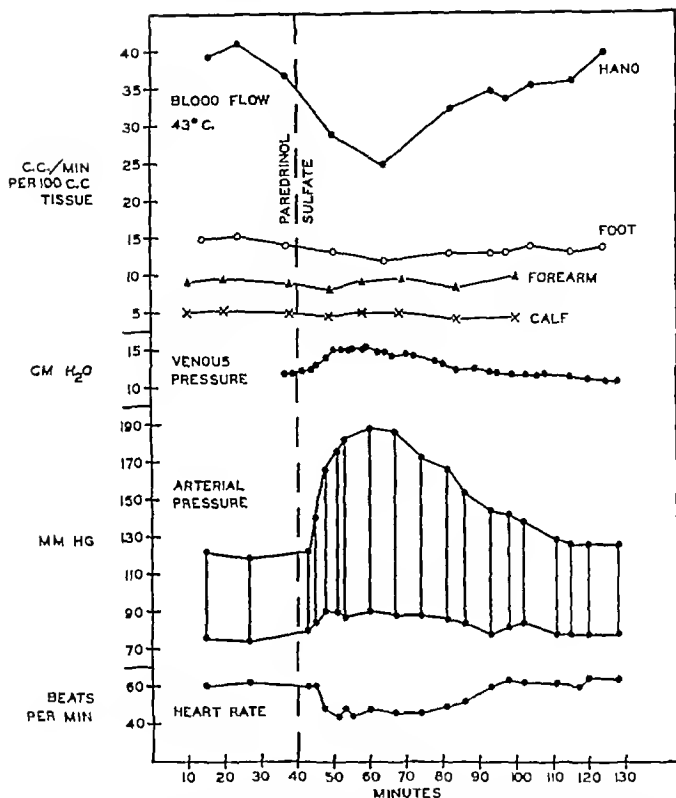


FIG. 1 EFFECT ON HEART RATE, ARTERIAL PRESSURE, AND VENOUS PRESSURE IN A NORMAL SUBJECT OF THE INTRAMUSCULAR INJECTION OF 35 MGm OF PAREDRIOL. EFFECT ON THE BLOOD FLOW IN HAND FOOT FOREARM AND CALF IN THE SAME SUBJECT OF 25 MGm OF THE SAME DRUG

spinal fluid pressure rose from 170 mm to 230 mm of water

Symptoms and signs Palpitation which was experienced by all the subjects was usually the only symptom noted. The drug caused no pain at the site of injection. There was no evidence of excitement, cerebral stimulation or tremor. The color of the hands and face did not change. The force of the apex impulse was greatly increased. The arterial pulsations in the neck were quite marked. Pistol shots were occasionally

heard in the femoral vessels. No disturbances in cardiac rhythm were observed. Three of the subjects complained of occipital headaches at pressures of 180-214 and 220 mm systolic and at 100-110 and 150 mm diastolic. One subject complained of mild precordial discomfort.

Electrocardiograms and phonocardiograms In 4 normal subjects the electrocardiographic tracings showed no change except in the T-waves (Figure 2). The T waves were usually increased by from 1 to 2 mm in height in Leads 1, 2, and

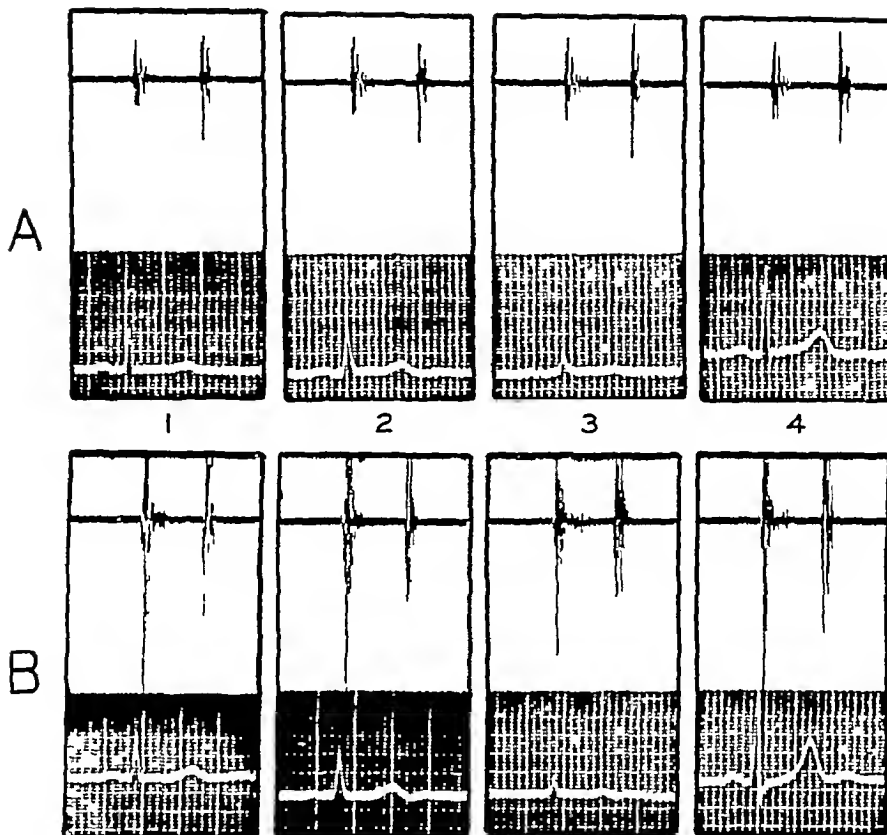


FIG 2A SIMULTANEOUS ELECTROCARDIOGRAM AND PHONOCARDIOGRAM OF A NORMAL RESTING SUBJECT

FIG 2B SIMULTANEOUS ELECTROCARDIOGRAM AND PHONOCARDIOGRAM OF THE SAME SUBJECT AFTER THE INTRAMUSCULAR INJECTION OF 25 MGm OF PAREDINOL.

3, although this change was not uniform and at times occurred in only one or two of these leads. In Lead 4 the T-waves were consistently higher, the average increase being 5 mm. In 3 cases with inverted T-waves in certain leads, the T-waves never became any deeper, usually they became less inverted and at times they became upright. Phonocardiograms (Figure 2) illustrated the great increase in the intensity of the heart sounds.

Blood flow, vasomotor reactions, and venous tone. In 5 subjects the blood flow in the hand at 43° C showed a definite, though not marked, fall after the intramuscular administration of 25 mgm of paredrinol, the flow dropped from an average of 34 cc to an average of 27 cc per minute per 100 cc of tissue. In 2 subjects the hand flow at 37° C also showed a moderate decrease. No significant change in the blood flow

in the foot was demonstrable when measured at 43° C (4 subjects), at 37° C (2 subjects) and at 32° C (1 subject). In 3 subjects the blood flow in the forearm and calf at 43° C showed no change. The spontaneous fluctuations in vasomotor tone became much less marked in the hand after administration of paredrinol, while in the foot there was only a slight decrease in vasomotor activity. Typical vasoconstriction (22), however, was obtained in both organs with such stimuli as a deep inspiration or pinching the skin. The venous tone was definitely increased in the 4 subjects tested. Two of these were normal subjects while the other 2 had had preganglionic sympathectomies. The increase in venous tone was of about the same magnitude as that observed following the administration of epinephrine. When paredrinol was pricked into the skin or injected

TABLE I
Effect of paredrolinol on the cardiac output (acetylene method)

Subject and age	Date	Blood pressure	Pulse rate	Oxygen consumption	Basal metabolic rate	Arterio-venous oxygen difference	Cardiac output			
							liters per minute	cc. per beat	liters per 100 cc. of oxygen consumed	liters per square meter of surface area
years	1939	mm Hg	per minute	cc. per minute	per cent	cc. per liter				
A. Y 26	February 3	120/86	62	219	-17.5	58.1 56.0	3.84	62.0	1.75	1.98
	February 3	200/116	50	243	-7.2	60.8 63.7	3.91	78.2	1.61	2.02
	February 3	194/116	54			59.0 58.4	4.14	76.6	1.70	2.13
E. A. S 30	February 27	112/78	60	218	-24.0	82.9 85.6	2.59	43.2	1.19	1.24
	March 8	182/86	52	253	-10.0	83.0 88.4	2.95	56.7	1.17	1.41

intracutaneously in concentrations of 1 to 40 a wheal surrounded by a flare was produced, which was usually attended by itching.

Cardiac output circulation time and basal metabolism. The cardiac output was determined in 2 subjects with the blood pressure elevated to 200 mm and 194 mm systolic, and 116 mm and 86 mm diastolic, respectively (Table 1). In neither subject was there a rise in minute output of the heart though as the stroke volume increased the heart rate decreased. The basal metabolic rate was measured 7 times in 3 subjects with an average increase of 7 per cent above the basal level. The histamine circulation time was measured 3 times in 2 subjects at the height of the blood pressure response and in neither subject did it differ from the values obtained at normal blood pressure levels.

Atropine. Three subjects were given from 3 to 4 mgm of atropine subcutaneously (Figure 3). The heart rate increased from an average of 71 to an average of 117 beats per minute. After the rate had become constant 2 of the subjects were given 25 mgm and the third 35 mgm of paredrolinol intramuscularly. The heart rate increased to an average of 135 beats per minute and the blood pressure reached an average height of 205 mm systolic and 130 mm diastolic. In these subjects the blood flow in the hand and foot at 43° C and in the foot at 40° C showed no significant change.

Posture. In normal subjects the high arterial blood pressure produced by paredrolinol showed little change when the subject was tilted from the horizontal position to an angle of from 50 to 75 degrees above the horizontal. The oral administration of 3 grains of sodium nitrite had no effect on the development of the paredrolinol hypertension in the horizontal position but the arterial blood pressure fell rapidly to either normal or subnormal levels when the subjects were tilted to the upright position (Figure 4).

Arteriosclerotic gangrene and preganglionic sympathectomy. In 2 subjects with early arteriosclerotic gangrene of the lower extremities and without hypertension the systolic blood pressures were maintained at levels of 186 and 200 mm. respectively, for at least 1 hour no change in skin temperature was observed. In 2 subjects with preganglionic sympathectomies the decrease in hand flow produced by paredrolinol was greater than that in the normal subjects.

Comparison of the effect of paredrolinol and paredrine (β -4 hydroxyphenylisopropylamine) on the cardiovascular system. Three of the above subjects were given 20 mgm of paredrine hydrobromide intramuscularly. Their arterial blood pressures rose to an average of 176 mm systolic and 91 mm diastolic, the heart rate slowed from an average of 66 to an average of 49 beats per minute. The subjects experienced no pain at the site of injection but they were soon aware of

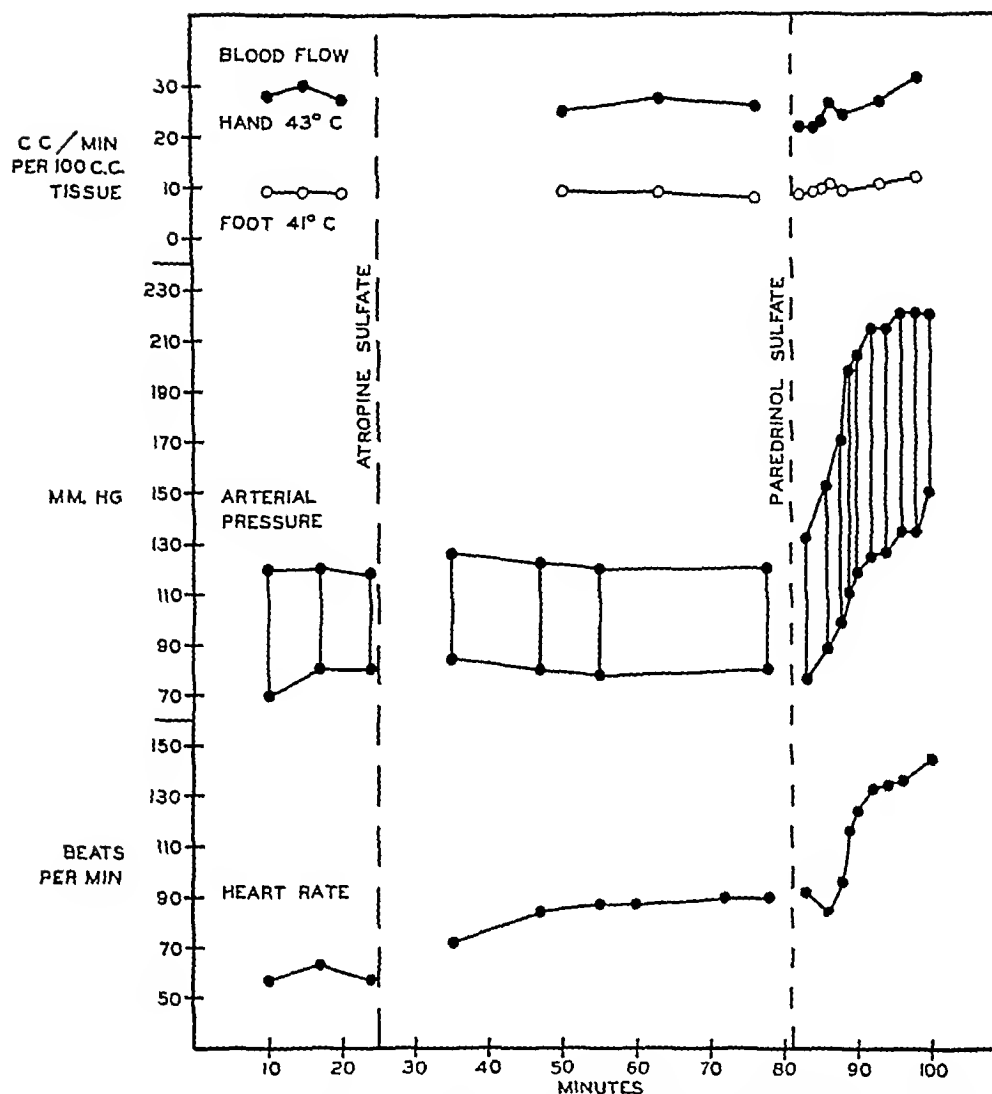


FIG 3 THE EFFECT OF INTRAMUSCULAR INJECTION OF 25 MG/M OF PAREDROL IN A NORMAL SUBJECT WHO HAD PREVIOUSLY RECEIVED 4 MG/M OF ATROPINE SUBCUTANEOUSLY

palpitation. No change in color of their skin was observed. The effect produced on the blood pressure and heart rate of these 3 subjects by the administration of 20 mg/m of paredrine was similar in nature and intensity to that produced by 25 mg/m of paredrinol.

DISCUSSION

Paredrinol in normal subjects produces a type of hypertension in which the only outstanding abnormalities regularly observed are the forceful apex beat, loud heart sounds and the high arterial pressure itself. The heart rate is usually, but not always, slower than in normal subjects at rest.

Palpitation is the only symptom experienced by most of the subjects. Other changes in the circulation are detectable only if the resting values for the individual subjects are known. While the blood flow and vasomotor reactions in the hand are decreased after administration of the drug, they are still within the normal range. Likewise, while the elevation of the venous pressure is definite and easily demonstrated by frequent determinations, the rise is not enough to produce an abnormal venous pressure unless the resting venous pressure is close to the upper limits of normal. Occipital headache, resembling that described in clinical hypertension, occurs when the blood pres-

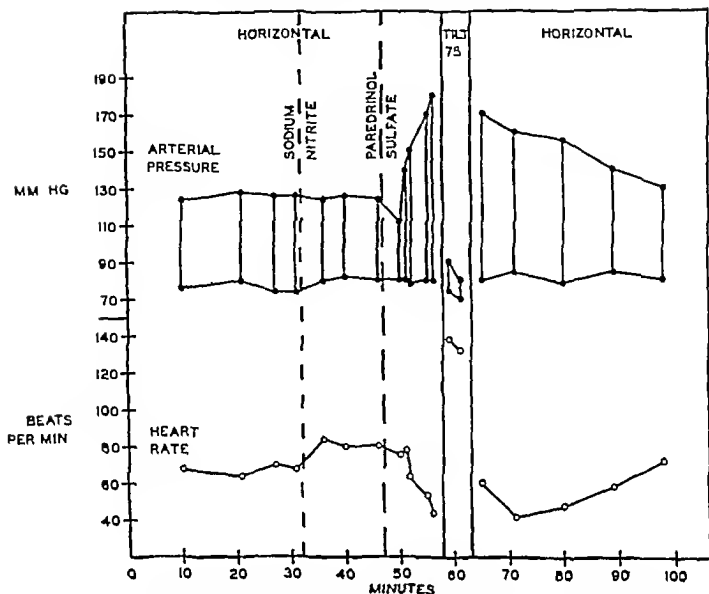


FIG. 4 THE EFFECT ON A NORMAL SUBJECT WITH PAREDROL HYPERTENSION OF THE ORAL ADMINISTRATION OF 3 GRAINS OF SODIUM NITRITE FOLLOWED BY TILTING TO THE UPRIGHT POSITION

sure is excessively elevated. The blood flow in the foot, forearm and calf shows no change, and the minute cardiac output remains unaltered. The decrease in heart rate is not the primary cause for the unchanged cardiac output and peripheral blood flow, for if the cardiac slowing accompanying paredrol administration is prevented by atropine the blood flow to the hand and foot does not become greater than the normal resting flow. Following the administration of paredrol the circulation time is unchanged. The basal metabolic rate is not affected significantly. Thus if a subject is seen shortly after the administration of the drug he presents a condition closely resembling clinical hypertension for typically in this condition except for the arterial blood pressure, all measurements of the various aspects of the circulation and of the other body functions are normal.

Hypertension induced by paredrol can be explained by two possible mechanisms either singly or combined. (1) Primary constriction of certain

portions of the minute vessels which regulate peripheral resistance (arterioles capillaries or venules) (2) primary venoconstriction and emptying of the vascular reservoirs, causing an increased venous return to the heart and a redistribution of the blood in the arterial and venous portions of the circulation. The observations recorded indicate that increased peripheral resistance does occur for in spite of the greatly increased arterial pressure head, blood flow through the tissues and cardiac output remain essentially normal. It does not necessarily follow however that the primary action of the drug is to cause such an increase in the peripheral resistance by a direct vasoconstrictor effect, for the constriction demonstrated may be a secondary response to an earlier primary change in the venous system. Evidence against the direct vasoconstrictor action of paredrol is the observation that, when the drug is injected or pricked into normal skin in concentrations as high as 1 to 40 a wheal surrounded by

flare results. The cutaneous wheal with the sensation of itching is not unlike that observed after the injection of histamine. This is in sharp contrast to the white spot formed by the intracutaneous injection of epinephrine. Too much emphasis, however, cannot be placed on the failure of paredrinol to produce visible vasoconstriction after intracutaneous injection, for the drug may act on the larger arterioles rather than on the capillaries or venules.

There is considerable evidence that the main action of paredrinol is on the venous reservoirs. Hypertension may therefore result from an increase in the arterial circulating blood volume because of a change in the distribution between the arterial and venous parts of the vascular bed. Rein believes that his experiments on dogs have demonstrated this to be the main factor in the production of the hypertension. In man, the venous pressure rises by from 30 to 40 mm of water while the arterial pressure is increasing. The venous tone in the extremities, as demonstrated by plethysmographic measurements, is also increased. The important effect of the quantity of circulating blood on this type of hypertension is clearly shown in postural experiments after the oral administration of 3 grains of sodium nitrite. In subjects whose venous systems respond to sodium nitrite with a decrease in venous tone, the drug has no measurable effect on the paredrinol hypertension while the body is in the horizontal position, when the upright position is assumed, however, the blood pressure falls from hypertensive levels either to normal or greatly subnormal levels. When the horizontal position is resumed, the blood pressure immediately returns to the hypertensive level which existed before tilting. The venous pooling induced by the combination of sodium nitrite and the upright position so reduces the circulating blood volume that the arterial blood pressure falls sharply. Thus, in these experiments with sodium nitrite, specific antagonistic changes are induced, hence the paredrinol hypertension is abolished. This is also of great practical importance because it offers a method of controlling the hypertension induced by paredrinol if the blood pressure rises to alarming heights or if a headache develops. The blood pressure is instantly lowered by amyl nitrite in the horizontal position and the headache quickly

disappears. The effect, however, lasts for only a minute. By tilting the subject upright the effect is greatly prolonged and a normal pressure can be maintained without difficulty.

On *theoretical* grounds one can account for the development of the hypertension induced by paredrinol by assuming a decrease in volume of the venous reservoirs and veins. This produces an increased venous return to the heart. As far as is known, the blood flow in the tissues is regulated chiefly by the requirements for metabolism and for heat conservation and dissipation. If the blood flow through the tissues is increased momentarily beyond these requirements by a rise in cardiac output resulting from the increased venous return, the vessels may well contract sufficiently to restore the blood flow to the normal level. Under such circumstances an initial increase in cardiac output will produce hypertension. The rise in blood pressure will tend to increase the work of the heart and reduce the cardiac output. When equilibrium is reached the increased arterial blood pressure will have reduced the cardiac output to normal and the tissue blood flow will also be at the normal level. As yet, sufficient evidence has not accumulated to determine whether the hypertension caused by paredrinol is produced (1) by the direct action of the drug on the small vessels controlling the peripheral resistance, or (2) by the direct action of the drug on the veins and venous reservoirs, causing primarily an increased venous return to the heart and a secondary increase in peripheral resistance. Neither of these mechanisms is necessarily antagonistic and both may play a part in the production of the hypertension.

The slow heart rate frequently encountered after the administration of paredrinol is caused by stimulation of the carotid sinus and aortic nerves. After atropine the fall in cardiac rate no longer occurs. If the subject is completely atropinized and the pulse allowed to become constant, paredrinol causes a further distinct rise in pulse rate. When the vagus is active, however, it masks the direct stimulating effect of paredrinol on the heart rate. After the administration of atropine, paredrinol causes a greater and more prolonged rise in arterial blood pressure, particularly in the diastolic level. This great rise in diastolic pressure (up to 150 mm Hg) is

caused by the marked increase in heart rate. Diastole becomes very short and consequently there is not sufficient time for the pressure to fall to the usual diastolic level before the next systole occurs. If the blood flow were appreciably increased, the diastolic pressure would not rise so steeply. Blood flow determinations on the hand and foot indicate that the peripheral resistance merely increases as the pressure rises and that the blood flow is not increased.

In view of the conclusion drawn by Rein from animal experiments that paredrolinol increases the blood pressure chiefly by acting in the veins and venous reservoirs, it was hoped that by increasing the pressure head the blood flow could be increased in the extremities in cases of arteriosclerotic gangrene without hypertension. The experiments with atropine and paredrolinol had previously demonstrated that the blood flow could not be increased through the normal hand and foot by raising the blood pressure to great heights. In the presence of incipient gangrene, however, it was possible that enough dilating substances would be present locally to maintain vasodilatation and permit an increase in flow. Skin temperature studies on 2 such cases revealed no change after the administration of paredrolinol. Similar results have been reported in cases of Buerger's disease in which hypertension was induced by paredrolinol (13). The possibility still remained that the increased peripheral resistance resulted from central stimulation of the vasoconstrictor nerves. That this was not the case was demonstrated by finding that the blood flow was slowed to an even greater degree in the sympathectomized than in the normal hand.

SUMMARY AND CONCLUSIONS

1 Paredrolinol (α N-dimethyl p-hydroxyphenethylamine) produces in normal subjects a type of acute arterial hypertension that closely resembles that observed in disease. The tendency to a slower heart rate, the vigorous apex impulse, the loud heart sounds, and the hypertension itself are the only outstanding abnormalities produced by the administration of the drug.

2 This hypertension differs greatly from that produced by epinephrine.

3 The arterial blood pressure response in different subjects, and in the same subject on dif-

ferent days, varies greatly. The average duration of the hypertension after the intramuscular injection of 25 mgm of paredrolinol is 1 hour.

4 The blood flow in the dilated hand is moderately decreased. The spontaneous fluctuations in vasomotor tone in the hand and foot are decreased. The venous tone in the hand is increased. The venous pressure is increased by from 30 to 40 mm. of water. The T waves in the electrocardiogram become higher. These changes are usually not great enough to be detectable unless the resting values for the particular subject are known.

5 There is no significant change in blood flow in the foot, forearm, and calf. The cardiac output, circulation time, and basal metabolism are not significantly altered.

6 The decrease in heart rate results from an increase in vagal tone brought about by stimulation of the carotid sinus and aortic nerves since if the vagal effect is removed by atropine paredrolinol causes an increase rather than a decrease in heart rate. When atropine is given before the injection of paredrolinol the arterial pressure, particularly the diastolic, rises to higher levels than after paredrolinol alone.

7 The combination of nitrite and tilting to the upright position pools sufficient blood to reduce the paredrolinol hypertension to normal. Thus if the arterial blood pressure rises to alarming heights or if headache develops, the hypertension can be rapidly and permanently reduced.

8. The peripheral blood flow in subjects with arteriosclerosis and in subjects who have had a preganglionic sympathectomy is not increased by raising the arterial pressure head with paredrolinol.

9 The hypertension produced by paredrolinol may result from either or both of the following mechanisms: (1) A primary increase in peripheral resistance from a direct vasoconstrictor effect on the minute vessels (arterioles, capillaries, venules). (2) a primary increase in venous tone and an emptying of the splanchnic reservoirs causing increased venous return to the heart and a secondary increase in peripheral resistance.

We wish to express our appreciation to Dr. Reno Porter and Dr. E. C. Eppinger for determinations of the cardiac output, and to Dr. Soma Weiss for helpful guidance and criticism of this work. The investigation was carried out with the technical assistance of Miss Sophia M. Simmons. S. B.

BIBLIOGRAPHY

- 1 Rein, H., Die physiologischen Grundlagen für die Wirkungsweise der Versuchssubstanz "Knoll H 75" *Klin. Wchnschr*, 1937, 16, 700
- 2 Rein, H., Über die Kreislauf- und Stoffwechselwirkungen des β -(p-Oxyphenyl)-Isopropyl-Methylamins *Arch. f exper Path. u. Pharmacol*, 1937, 187, 617
- 3 Heymans, C., and Bayless, F., Sur l'action circulatoire de la β -p-oxyphenyl-isopropyl-méthylamine. *Arch. internat. de pharmacodyn. et de therap*, 1937, 56, 319
- 4 Lindner, W., Über die pharmakologische Wirkung des β -(p-Oxyphenyl) isopropylmethylamins (Veritol, Präparat H 75) *Arch. f exper Path. u. Pharmacol*, 1937, 187, 444
- 5 Schneider, D., Klinische Erfahrungen mit dem Kreislaufmittel "Veritol" *Klin. Wchnschr*, 1937, 16, 736
- 6 Robbers, H., "Veritol" ein neues, stark wirksames Kreislaufmittel *München. med. Wchnschr*, 1937, 84, 819
- 7 Grosse-Brockhoff, F., and Kaldenberg, F., Klinische Untersuchungen über die kreislaufwirksame Substanz H 75 (Veritol) *Klin. Wchnschr*, 1937, 16, 948
- 8 Schneider, H., and Kopp, H., Untersuchungen über die kreislaufwirksame Substanz H 75 ("Veritol") bei chirurgischen Erkrankungen *Klin. Wchnschr*, 1937, 16, 1672
- 9 Schöndorf, T., Klinisch-experimentelle Untersuchungen mit dem neuen Kreislaufmittel Veritol *München. med. Wchnschr*, 1938, 85, 333
- 10 Klostermeyer, W., and Jonsson, B., Klinische Untersuchungen über das neue Kreislaufmittel "Veritol" mit bemerkswerten Ekg-Befunden. *Klin. Wchnschr*, 1937, 16, 1724
- 11 Meyer, F., and Spiegelhoff, W., Die Wirkung des Veritols auf die Zirkulationsgrößen des gesunden Menschen *Klin. Wchnschr*, 1937, 16, 1342
- 12 Grosse-Brockhoff, F., and Kaldenberg, F., Über den Antagonismus von Sympathicus und Vagus unter der Einwirkung adrenalinähnlicher Substanzen. *Arch. f exper Path. u. Pharmacol*, 1938, 188, 383
- 13 Abbott, W. O., and Henry, C. M., Paredrine (β -4-hydroxyphenylisopropylamine) A clinical investigation of a sympathomimetic drug *Am J M Sc.*, 1937, 193, 661
- 14 Freeman, N. E., The effect of temperature on the rate of blood flow in the normal and in the sympathectomized hand. *Am J Physiol*, 1935, 113, 384
- 15 Stead, E. A., Jr., and Kunkel, P., A plethysmographic method for the quantitative measurement of the blood flow in the foot. *J Clin. Invest.*, 1938, 17, 711
- 16 Kunkel, P., Stead, E. A., Jr., and Weiss, S., Blood flow and vasomotor reactions in the hand, forearm, foot and calf in response to physical and chemical stimuli. *J Clin. Invest.*, 1939, 18, 225
- 17 Grant, R. T., and Pearson, R. S. B., The blood circulation in the human limb, observations on the differences between the proximal and distal parts and remarks on the regulation of body temperature. *Clin. Sc.*, 1938, 3, 119
- 18 Capps, R. B., A method for measuring tone and reflex constriction of the capillaries, venules and veins of the human hand with the results in normal and diseased states *J Clin. Invest.*, 1936, 15, 229
- 19 Moritz, F., and von Tabora, D., Über eine Methode, beim Menschen den Druck in oberflächlichen Venen exakt zu bestimmen. *Deutsches Arch. f klin. Med.*, 1909-10, 98, 475
- 20 Grollman, A., Friedman, B., Clark, G., and Harrison, T. R., Studies in congestive heart failure. XXIII. A critical study of methods for determining the cardiac output in patients with cardiac disease. *J Clin. Invest.*, 1933, 12, 751
- 21 Weiss, S., Robb, G. P., and Blumgart, H. L., The velocity of blood flow in health and disease as measured by the effect of histamine on the minute vessels *Am. Heart J.*, 1929, 4, 664
- 22 Bolton, B., Carmichael, E. A., and Stürup, G., Vasoconstriction following deep inspiration. *J Physiol*, 1936, 86, 83

TREATMENT OF ADDISON'S DISEASE WITH DESOXY-CORTICOSTERONE ACETATE, A SYNTHETIC ADRENAL CORTICAL HORMONE (PRELIMINARY REPORT)¹

By GEORGE W. THORN, R. PALMER HOWARD² AND KENDALL EMERSON JR.

(From the Chemical Division, Medical Clinic, Johns Hopkins University and Hospital, Baltimore)

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The successful preparation of extracts of the adrenal cortex which possessed the property of maintaining adrenalectomized animals (1, 2, 3) stimulated attempts to isolate the substance or substances responsible for this activity. Reichstein has pointed out in his review article (4) that, thus far, several steroid compounds have been isolated from adrenal cortical extracts by Kendall, Reichstein, Wintersteiner, Pfaffner, and Grollman. At least 3 of these compounds have been shown to be capable, on injection, of maintaining adrenalectomized animals in good condition (4).

The small quantity of active material present in the adrenal cortex and the necessarily limited supply of adrenal glands restrict greatly the quantity of crystalline material which may be obtained from this natural source. The use of crystalline compounds would be of distinct advantage in the treatment of patients with Addison's disease because of the practical difficulties encountered in attempting to prepare extracts of uniform potency. It appears that a synthetic preparation offers the most likely possibility of obtaining an adequate quantity of crystalline material possessing "cortin-like" properties.

In 1937 Steiger and Reichstein (5) announced the preparation of desoxy-corticosterone acetate (Δ^4 pregnene 21-ol 3, 20-dione, acetate) from stigmasterol. Recently, Reichstein and von Euw (6) have succeeded in obtaining desoxy-corticosterone from an extract of beef adrenals, thus establishing the natural occurrence of this compound. The effectiveness of desoxy-corticosterone acetate (synthetic) in maintaining bilaterally adrenalectomized dogs (7, 8) prompted us to investigate the possibility of using this compound

in the treatment of patients with Addison's disease.

In this report we have studied the effect of subcutaneous or intramuscular injections of a solution of desoxy-corticosterone acetate³ in oil in 8 patients with Addison's disease. Because of the known beneficial effect of added sodium salts (9, 10) on the course of the disease, particular care was taken to limit the sodium chloride intake of the patients during these studies. However, in 2 of the patients (C.N. and J.Z.) it was considered advisable to undertake desoxy-corticosterone acetate treatment, while these patients were receiving sodium chloride therapy. Subsequently, it was possible to discontinue completely the additional sodium chloride therapy during the course of treatment with desoxy-corticosterone acetate.

No attempt was made to reduce the daily intake of potassium (11) in any of the patients. It is obvious that a diet of low sodium chloride and normal or high potassium content is not advocated for clinical application but is desirable for critical evaluation of the effectiveness of a compound suspected of possessing "cortin-like" activity. It is also apparent that the patient's requirement of hormone on such a regimen will be much greater than under conditions in which the diet is supplemented by the addition of sodium salts, or restricted in potassium content.

METHODS

All of the patients were studied on the metabolism ward of the Johns Hopkins Hospital. For the purpose of balance studies each patient was provided with a constant diet and a constant fluid intake, the same items of food being ingested each day throughout the period of investigation. A quantity of food sufficient for a 5-day period was purchased at one time and an aliquot

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² Jacques Loeb Fellow in Medicine.

³ The synthetic desoxy-corticosterone acetate (Per-corten) used in this study was supplied by Messrs. Ciba through the courtesy of Doctors K. Miescher and E. Oppenheimer.

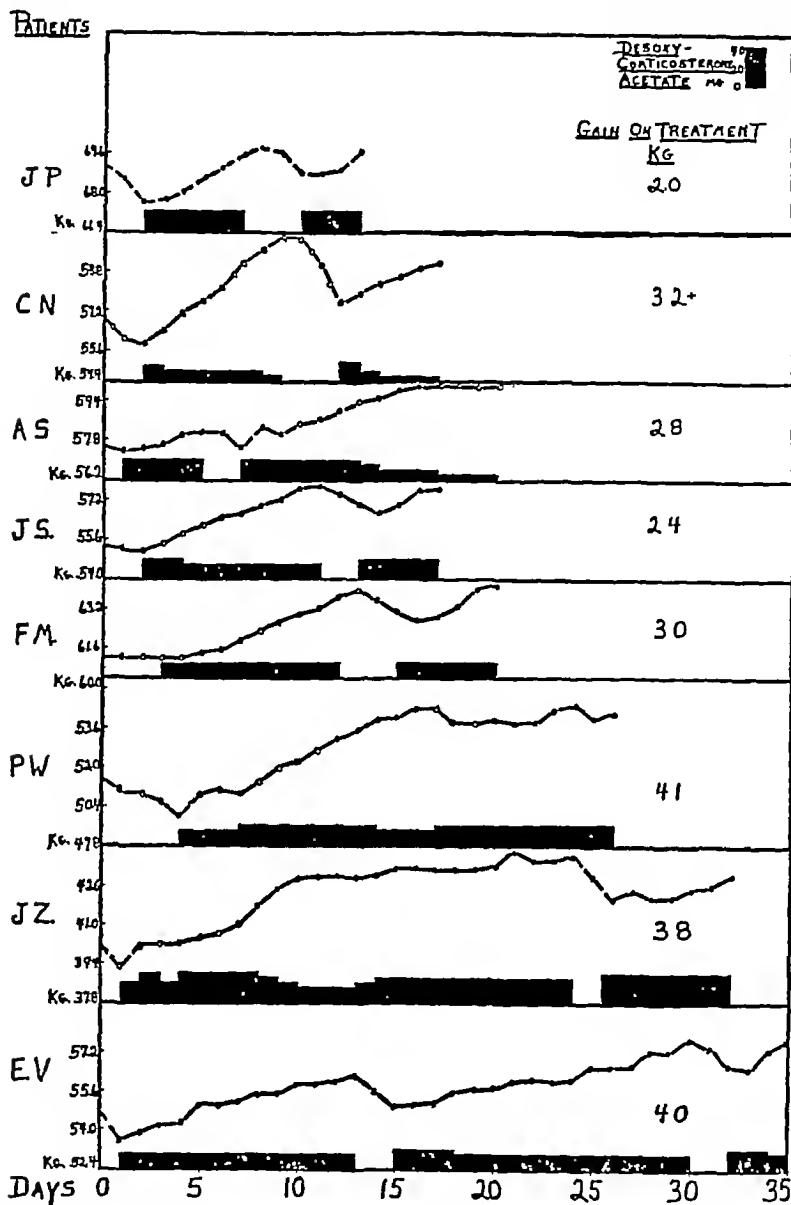


FIG 1 THE EFFECT OF DESOKY-CORTICOSTERONE ACETATE TREATMENT ON THE BODY WEIGHT OF PATIENTS WITH ADDISON'S DISEASE

of one day's entire diet was analyzed for its mineral content. Since the food was prepared without added seasoning, each patient was permitted an accurately weighed quantity of sodium chloride (3 grams daily) which was added to the food immediately before being ingested.

At 10 p.m. each evening the patients voided and were then weighed. No food or fluid was permitted during the night. At 7 a.m. before arising, the blood pressure was determined. The patients then voided (completing the 24-hour period) and were weighed. By this method it was possible to obtain an approximate value for the rate of insensible water loss during a 9-hour period.

Specimens of blood for chemical analyses were withdrawn under oil and without stasis from the antecubital vein, the patients having been fasted for 15 hours previously. The following determinations were made: hematocrit, serum concentration of sodium (12), chloride (13) and potassium (14, 15), carbon dioxide combining power of the serum (16), blood nonprotein nitrogen (17) and blood sugar (Folin-Wu).

Plasma volume measurements were made according to the technic of Gregersen and Gibson (18, 19) using the dye, T-1824. Duplicate samples of blood for serum were withdrawn at intervals of 20 minutes for 2 hours following the injection of the dye. The concentration

of the dye in the samples of serum was determined by means of the Evelyn photoelectric colorimeter (20 21)

Twenty four-hour urine specimens were collected, preserved with toluol and later analyzed for sodium (12) chloride (13) potassium (14 15) inorganic phosphorus (22) and total nitrogen (macro-Kjeldahl) content

Following a control period, the patients were treated with subcutaneous injections of a solution of desoxy corticosterone acetate in oil. Later desoxy-corticosterone acetate treatment was discontinued for a period of from 24 to 72 hours. The length of the withdrawal period was determined by the patient's clinical condition. Subsequently desoxy-corticosterone acetate treatment was resumed. During each of these periods the patient's clinical condition, body weight, blood pressure, plasma volume, plasma concentration, and renal excretion of electrolytes were observed. To control the possible psychological effect of discontinuing and resuming treatment, subcutaneous injections of a placebo were substituted for injections of hormone during the withdrawal periods.

OBSERVATIONS

The specific changes which occurred as the result of desoxy-corticosterone acetate treatment are recorded in the charts and tables. A more detailed account of the effect of treatment may be found in the résumé attached to the individual protocols

Body weight

A change in body weight constitutes one of the most sensitive indicators of a change in the clinical status of patients with Addison's disease. Improvement in clinical condition is regularly accompanied by weight gain and a relapse is associated with weight loss

In every patient treatment with desoxy-corticosterone acetate resulted in a marked gain in body weight which paralleled clinical improvement (Figure 1). A significant increase in weight was observed within 48 hours after treatment was begun. Withdrawal of treatment resulted in a prompt and progressive decrease in weight which was associated with decreased muscular efficiency loss of appetite and the onset of symptoms of adrenal insufficiency. Institution of treatment at this time prevented further weight loss and within a period of 48 hours resulted in weight gain. These sudden changes in body weight appeared to be directly related to changes in mineral and water balance, weight gain being invariably associated with a retention of sodium chloride and water and weight loss usually being

accompanied by a diuresis and increased renal excretion of sodium and chloride. Occasionally, a slight decrease in body weight and a diuresis were noted on the first or second day of treatment despite a retention of sodium and chloride. This unexpected decrease in weight resulted from the marked potassium diuresis occasioned by the institution of treatment

The diet of constant mineral and caloric content limited to some extent the total weight gain which was possible during the experimental period, and the weight increments tabulated in Figure 1 were modified by the weight loss which occurred during the withdrawal periods

Blood pressure

A change in blood pressure usually accompanies a significant change in the clinical status of patients with Addison's disease although frequently a considerable delay in blood pressure response is observed both during remission and relapse. A rapid increase in blood pressure from shock level is usually noted in patients in crisis following adequate treatment with infusions of sodium chloride and glucose, or adrenal cortical extract, or following a combination of both forms of therapy. The

TABLE I
The effect of desoxy-corticosterone acetate treatment on the blood pressure of patients with Addison's disease

Patients	Blood pressure*		
	Previous to treatment	During treatment	Treatment
J S	96/72	133/80	10 mgm daily for 120 days
E W	94/65	130/80	10 mgm daily for 150 days
C N	95/59	110/70	3 mgm daily for 40 days
J Z	93/59	106/66	25 mgm daily for 20 days
P W	92/61	103/78	20 mgm daily for 20 days
A. S.	105/66	114/70	10 mgm daily for 30 days
J P †	129/91	133/81	20 mgm daily for 8 days
F M	92/60	112/70	15 mgm daily for 90 days

* All blood pressure measurements were made at 7 a m with the patients resting quietly in bed. The values in the table represent the average of 3 determinations made on 3 successive days during the period noted

† These values represent the blood pressure level at the time desoxy-corticosterone acetate treatment was substituted.

‡ These values represent the measurements obtained during a period in which the patients were being maintained on desoxy-corticosterone acetate, without added sodium chloride therapy

§ This patient was known to have antedated the onset of symptoms of

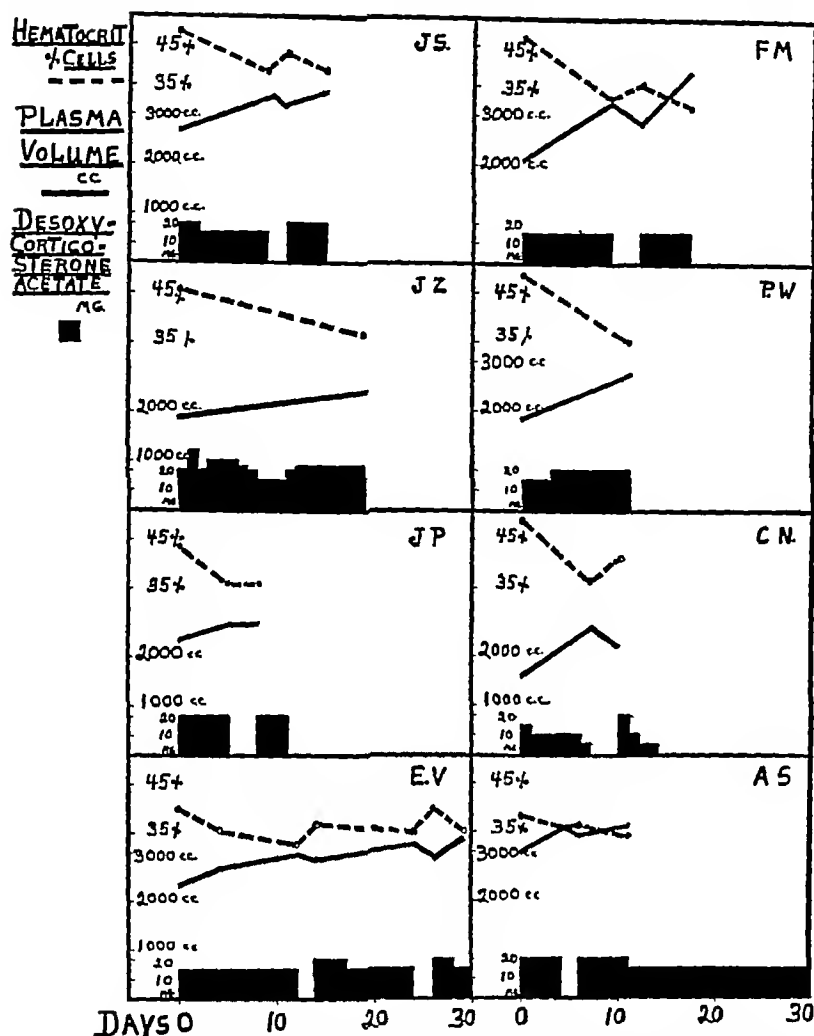


FIG 2 THE EFFECT OF DESOXY-CORTICOSTERONE ACETATE TREATMENT ON THE PLASMA VOLUME AND HEMATOCRIT OF PATIENTS WITH ADDISON'S DISEASE

difficulty, subsequently, with which the blood pressure is raised to a normal level and maintained at that level is well known

Treatment with desoxy-corticosterone acetate (3 to 25 mgm daily) for periods of from 8 to 50 days resulted in increased blood pressure in all of the patients (Table I). In 1 patient (F.M.) no increase in blood pressure was observed until treatment had been continued for more than 20 days. In all instances an increase in blood pressure was associated with increased strength, improved appetite, and sense of well-being

Pulse rate and body temperature

Treatment with desoxy-corticosterone acetate resulted in no appreciable change in either the

pulse rate or body temperature in 6 of the patients. In 2 patients, continued daily injections of 20 to 25 mgm of desoxy-corticosterone acetate in sesame oil (4 to 5 cc of oil daily) resulted, after 15 to 20 days of treatment in an increased rectal temperature (101° to 102° F) and in tachycardia. Withdrawal of treatment was accompanied by a rapid return of temperature and pulse rate to normal. Subsequently, injections of the same quantity of desoxy-corticosterone acetate in sesame oil (4 to 5 cc) again resulted in a febrile response. The intradermal injection of sesame oil alone was followed by a very marked local skin reaction in these patients. In control subjects, a negative skin reaction was observed with the same quantity of oil. Other patients

TABLE II

The effect of desoxy-corticosterone acetate on the total plasma content of sodium chloride, and potassium

Patient	Before treatment			During treatment			Withdrawal of treatment 48 to 72 hours			Treatment restored		
	Plasma level	Plasma volume	Total plasma content	Plasma level	Plasma volume	Total plasma content	Plasma level	Plasma volume	Total plasma content	Plasma level	Plasma volume	Total plasma content
J S.	Na 138.5 Cl 96.4 K 6.5	2730	Na 378.0 Cl 263.2 K 177	Na 141.8 Cl 102.0 K 61	3370	Na 476.8 Cl 343.9 K 20.6	Na 140.4 Cl 97.6 K 7.1	3240	Na 455.0 Cl 316.1 K 22.9	Na 142.7 Cl 95.8 K 6.7	3440	Na 490.8 Cl 329.6 K 23.1
E. V	Na 134.8 Cl 101.2 K 4.2	2720	Na 367.0 Cl 275.2 K 11.4	Na 142.7 Cl 100.6 K 5.6	3150	Na 449.0 Cl 316.9 K 17.8	Na 137.4 Cl 96.8 K 4.9	2950	Na 405.5 Cl 285.4 K 14.5	Na 139.3 Cl 100.4 K 4.9	3240	Na 451.5 Cl 325.3 K 15.9
F M	Na 135.6 Cl 92.8 K 6.7	2085	Na 282.7 Cl 193.7 K 13.9	Na 136.7 Cl 97.8 K 4.2	3300	Na 450.1 Cl 322.4 K 13.8	Na 137.4 Cl 96.4 K 6.3	2890	Na 397.5 Cl 278.5 K 18.1	Na 139.2 Cl 101.2 K 6.7	3900	Na 543.5 Cl 394.8 K 26.0
A S	Na 135.2 Cl 97.2 K 6.2	2585	Na 350.0 Cl 251.0 K 16.1	Na 141.0 Cl 100.4 K 7.1	3500	Na 493.6 Cl 351.4 K 24.5	Na 135.2 Cl 101.0 K	3380	Na 457.1 Cl 341.4 K	Na 139.7 Cl 102.2 K 7.8	3400	Na 475.0 Cl 347.5 K 26.5
C. N	Na 131.4 Cl 95.0 K 5.0	1620	Na 213.2 Cl 154.0 K 8.1	Na 136.9 Cl 102.6 K 5.9	2540	Na 348.0 Cl 260.5 K 15.0	Na 134.2 Cl 99.8 K 5.9	2200	Na 295.3 Cl 219.6 K 12.9	Na 138.2 Cl 104.4 K 6.6	2280	Na 315.1 Cl 238.0 K 15.0
J P	Na 133.5 Cl 96.4 K 4.4	2390	Na 318.8 Cl 230.1 K 10.6	Na 139.6 Cl 103.6 K 5.2	2710	Na 378.3 Cl 280.8 K 14.2	Na 128.7 Cl 102.2 K 5.2	2780	Na 358.0 Cl 284.0 K 14.6			
P W	Na 127.3 Cl 90.6 K 6.1	1865	Na 237.8 Cl 169.0 K 11.8	Na 139.3 Cl 103.8 K 5.0	2770	Na 386.8 Cl 287.6 K 14.0						
J Z.	Na 131.2 Cl 91.8 K 6.1	1900	Na 249.0 Cl 174.3 K 11.6	Na 138.7 Cl 96.8 K 6.0	2330	Na 320.6 Cl 225.5 K 13.9						

who had been injected with the same preparation but in whom no untoward symptoms had been observed were found to have either slightly positive or negative intradermal tests

Plasma volume

In a previous study (23) it was shown that an increase in plasma volume occurred in patients with Addison's disease following treatment with adrenal cortical extract. It is well known that a reduction in plasma volume with consequent hemoco-concentration regularly accompanies a relapse.

Desoxy-corticosterone acetate treatment was associated with a marked increase in plasma volume (350 to 1,800 cc.) in all of the patients (Figure 2 and Table II). Withdrawal of treatment (48

hours or more) resulted in an appreciable reduction in plasma volume, resumption of treatment restored the plasma volume to normal. Changes in hematocrit corresponded closely to the changes in plasma volume.

Total blood volumes (Table III) were calculated from the plasma volumes and hematocrit determinations (19). Before treatment with desoxy-corticosterone acetate it was observed that the values for 5 of the patients were considerably lower than the values given by Gibson for normal individuals of the same surface area (24). Treatment with desoxy-corticosterone acetate resulted in a marked increase in the blood volume of these patients so that the values after treatment approximated normal. In one patient (F M,

TABLE III

The effect of desoxy-corticosterone acetate treatment on the total blood volume of patients with Addison's disease

Patient	Previous to treatment with desoxy-corticosterone acetate				During treatment with desoxy-corticosterone acetate			
	Surface area	Expected blood volume*	Actual blood volume	Per cent of normal	Surface area	Expected blood volume	Actual blood volume	Per cent of normal
	square meters	cc.	cc.		square meters	cc.	cc.	
J S	1.72	5050	5200	103	1.76	5200	5500	105
E V	1.60	4560	3740	80	1.67	4850	4940	102
F M	1.68	4900	3880	75	1.71	5000	5730	115†
A S	1.70	5000	4310	86	1.73	5100	5430	107
C N	1.61	4100	3180	75	1.67	4150	3960	95
J P	1.69	4180	4210	101	1.70	4200	4200	100
P W	1.49	4000	3600	90	1.54	4300	4200	97
J Z	1.38	3300	3460	105	1.44	3700	3640	98

* Calculated by the method of Gibson for normal individuals of the same surface area

† Refer to text (Plasma volume)

Figure 2) it was apparent that the optimum dose of desoxy-corticosterone acetate had been exceeded since his total blood volume increased to 115 per cent of the calculated normal. With a reduction in the dose of desoxy-corticosterone acetate the blood volume has returned to approximately 100 per cent of the expected normal.

Plasma electrolytes

Severe adrenal insufficiency is usually associated with a marked reduction in the plasma concentration of sodium and chloride and an increase in the concentration of potassium (9, 10). It has been noted (23) that treatment with adrenal cortical extract may result in considerable improvement in the clinical condition of patients with Addison's disease before an increase in the plasma concentration of sodium and chloride may be detected. However, a considerable increase in the total plasma content of sodium and chloride may be demonstrated at this time as a result of the increase in plasma volume (23).

Desoxy-corticosterone acetate treatment resulted in a restoration of the plasma sodium and chloride concentration to normal levels in all of the patients (Table II). It is interesting to note that in 1 of the patients (E V) no increase in plasma concentration of sodium or chloride occurred during the first week of treatment al-

though considerable clinical improvement was noted during this period (Figure 3). The total plasma content of sodium and chloride, however, was greatly increased as the result of increased plasma volume. In 1 patient a rise in the plasma concentration of sodium and chloride preceded a demonstrable increase in plasma volume. In this instance the total plasma content of sodium and chloride was increased as a result of the increased concentration of these ions. Improvement in the clinical condition of the patients was associated in every instance with an increase in the total plasma content of sodium and chloride whereas withdrawal of treatment and the subsequent development of signs of adrenal insufficiency were invariably associated with a reduction in the total plasma content of these ions.

Desoxy-corticosterone acetate treatment resulted in a reduction in the plasma concentration of potassium in those patients in whom the concentration of this ion was elevated. Changes in the total plasma content of potassium as a result of treatment with desoxy-corticosterone acetate were variable (Table II) inasmuch as the effect of the hormone in lowering the concentration of potassium was more than balanced by the increased plasma content resulting from the augmented plasma volume.

Sodium and chloride balance

It has been shown that in patients with Addison's disease, treatment with adrenal cortical extract results in a positive sodium and chloride balance (23). Withdrawal of adrenal cortical extract treatment is associated with a diuresis, an increased excretion of sodium and chloride and weight loss. Since in the absence of diarrhea or excessive sweating, approximately 95 per cent of ingested sodium and chloride is excreted in the urine, the renal excretion of these ions under these conditions may be used as a basis for calculating balance.

Treatment with desoxy-corticosterone acetate resulted in a marked retention of sodium in 7 of 8 patients (Figure 4, Table IV). Withdrawal of treatment resulted in a marked negative balance, and resumption of treatment resulted in sodium retention in all. Changes in chloride balance corresponded closely to the changes observed in sodium balance.

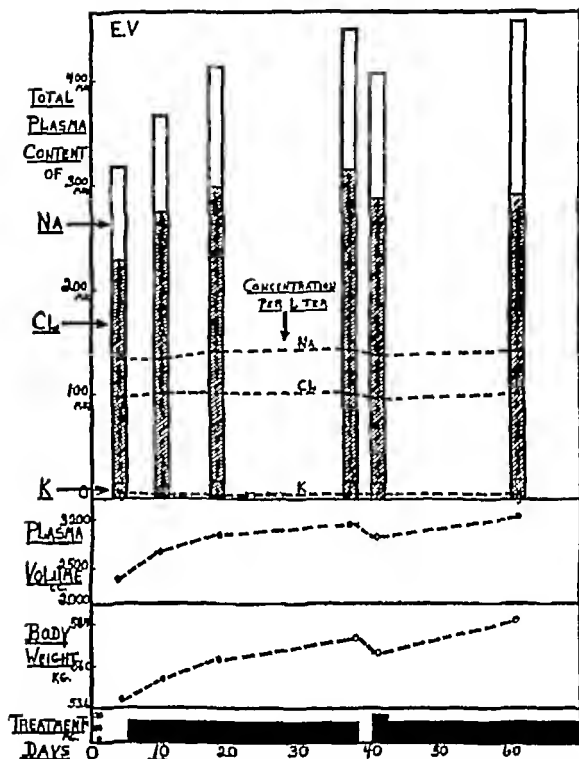


FIG. 3 THE EFFECT OF DESOXY-CORTICOSTERONE ACETATE TREATMENT ON THE CONCENTRATION AND TOTAL QUANTITY OF PLASMA SODIUM CHLORIDE, AND POTASSIUM*

* Refers to a single daily intramuscular injection of desoxy-corticosterone acetate in sesame oil.

Renal excretion of potassium inorganic phosphorus and total nitrogen

In all of the patients withdrawal of desoxy-corticosterone acetate treatment was associated with a decreased renal excretion of potassium. This effect was similar to that observed in patients treated with adrenal cortical extract (23). The average reduction in excretion following desoxy-corticosterone acetate treatment ranged from 4 to 20 m eq daily during the withdrawal period. In substitution of desoxy-corticosterone acetate treatment resulted in a marked increase in the renal

excretion of potassium during the first 2 to 4 days of treatment (7 patients). The average increase during this period ranged from 9 to 65 m eq per day. When desoxy-corticosterone acetate treatment was substituted for adequate adrenal cortical extract therapy no alteration in potassium excretion was noted.

Following desoxy-corticosterone acetate treatment changes in the excretion of inorganic phosphorus were similar to but not nearly so striking as the changes observed in potassium excretion. During the period of hormone withdrawal

TABLE IV

The effect of desoxy-corticosterone acetate treatment on sodium and chloride balance

Patient	Previous to treatment	During treatment	Withdrawal of treatment	Treatment restored
J S	m eq per day Na + 24	m eq per day +75.3	m eq per day - 19.7	m eq per day +38.9
	Cl + 3.5	+60.9	- 1.0	+23.7
E V	Na -123.8	+35.4	-129.4	+36.3
	Cl -128.8	+34.8	- 87.7	+37.4
F M	Na - 57.2	+47.9	- 70.6	+39.5
	Cl - 82.6	+ 5.7	- 97.7	- 4.3
A S	Na	+25.9	- 42.5	+53.4
	Cl	+28.9	- 28.3	+33.7
C N	Na - 67.6	+91.4	-112.2	+48.8
	Cl - 72.8	+92.0	- 99.0	+44.2
J P	Na - 17.3	+77.2	- 7.6	+37.8
	Cl - 32.8	+70.8	- 26.0	+42.8
P W	Na - 50.3	+63.4		
	Cl - 57.0	+37.6		
J Z	Na	- 2.3	- 63.1	+ 9.8
	Cl	- 5.0	- 48.0	+ 2.8

average decrease in inorganic phosphorus excretion ranged from 50 to 300 mgm per day (6 patients). During the first 4 days of treatment following a withdrawal period, an increased excretion of from 60 to 300 mgm daily of inorganic phosphorus was noted (7 patients).

Changes in the renal excretion of total nitrogen following desoxy-corticosterone acetate treatment were neither striking nor consistent.

Carbohydrate metabolism

The difficulty in obtaining satisfactory data (glucose tolerance test, insulin sensitivity, epinephrine response, and respiratory quotients) during the untreated control periods of patients with Addison's disease has hindered thus far any careful study of the effect of desoxy-corticosterone treatment on carbohydrate metabolism. Blood sugar determinations in the fasting state (8 patients) preceding treatment with desoxy-corticos-

terone acetate averaged 79 mgm per cent (73 to 88 mgm per cent), during the initial period of treatment with desoxy-corticosterone acetate an average value of 77 mgm per cent (58 to 88 mgm per cent) was observed, during a period of treatment (6 patients) the average value was 79 mgm per cent (64 to 92 mgm per cent), following the resumption of treatment (4 patients) the average blood sugar level was found to be 79 mgm per cent (63 to 96 mgm per cent).

In 2 of the patients (J Z and C N) the administration of 175 grams of glucose per kgm of body weight was followed by a flat blood sugar curve although the patients at that time appeared to be in good condition.

The blood sugar values following the administration of glucose orally to bilaterally adrenalectomized dogs treated with desoxy-corticosterone acetate and maintained on a diet of low sodium and chloride content were not significantly different from the values obtained in normal dogs maintained on a similar diet (25).

Blood nonprotein nitrogen

In adrenalectomized dogs the nonprotein nitrogen level of the blood is a sensitive indicator of a change in the animal's condition. In patients with Addison's disease, unless complicated by a renal lesion, a significant rise in blood nonprotein nitrogen is rarely observed unless accompanied by rather marked dehydration.

In the 8 patients studied in this report, the average blood level of nonprotein nitrogen upon instituting desoxy-corticosterone acetate treatment was found to be 34 mgm per cent (29 to 40 mgm per cent). Treatment with desoxy-corticosterone acetate resulted in no significant change.

Pigmentation

Except for the changes in color which resulted from increased hydration no alteration in pigmentation has as yet been observed in any of the patients following treatment with desoxy-corticosterone acetate (2 to 5 months).

Dosage and mode of administration

The desoxy-corticosterone acetate used in these studies was prepared in oil of sesame, 5 mgm of the compound being contained in 1 cc of oil. In-

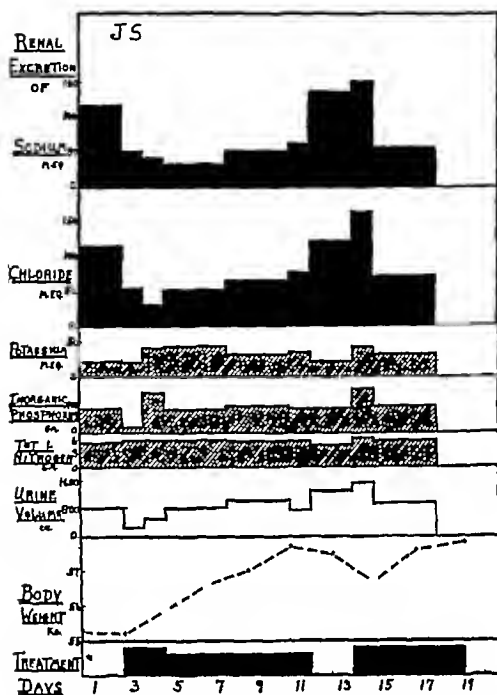


FIG. 4 THE EFFECT OF DESOXY-CORTICOSTERONE ACETATE TREATMENT ON THE RENAL EXCRETION OF ELECTROLYTES IN A PATIENT WITH ADDISON'S DISEASE*

jections were made subcutaneously or intramuscularly and usually once daily, since animal experiments (8) indicated that the material in oil solution has a prolonged action (24 hours). In most patients a single daily injection of 2 to 10 mgm daily was sufficient for maintenance when added sodium chloride therapy was employed. The withdrawal of sodium chloride therapy (10 to 20 grams of NaCl daily) increases the requirement of desoxy-corticosterone by approximately 10

mgm daily. A quantity as great as 30 to 35 mgm daily has been injected intramuscularly with no untoward effect.

The continued daily injection of more than 3 cc of sesame oil was attended by the development of fever, tachycardia and malaise in 2 patients. The withdrawal of treatment resulted in a prompt disappearance of these signs and symptoms. It was shown that in these patients this untoward reaction was due to the injection of sesame oil since the intradermal injection of oil alone resulted in a very marked positive skin reaction and in patient 10 a febrile response and since the subcutaneous implantation of 200 mgm of crystal line desoxy-corticosterone acetate was associated with no systemic or local reaction.

* Refers to a single daily intramuscular injection of desoxy-corticosterone acetate in sesame oil. During this study the patient was provided with a constant diet which was found to contain by analysis, 123.8 meq of sodium, 123.8 meq of chloride, 55.4 meq of potassium, 1.24 grams of total phosphorus and 7.37 grams of total nitrogen.

tients it was possible at a later date to maintain them successfully on injections of the same preparation of desoxy-corticosterone acetate in oil by resuming treatment with very small intramuscular injections and slowly increasing the daily dose. It is also possible to prepare desoxy-corticosterone acetate in a solution of peanut oil.

The prolonged action of the compound following its intramuscular injection permits effective therapy with a single daily injection. It should be noted that in the crisis of adrenal insufficiency immediate treatment with intravenous sodium chloride and glucose solution, and an aqueous preparation of adrenal cortical extract should accompany intramuscular injections of desoxy-corticosterone acetate in oil since several hours may be required for the absorption of the latter.

Studies thus far indicate that in both adrenalectomized dogs and patients with Addison's disease 1 mgm of a solution of desoxy-corticosterone acetate in oil is equivalent in effect to approximately 3 cc of a potent commercial adrenal cortical extract,⁴ under conditions in which the desoxy-corticosterone acetate in oil is administered as a single daily intramuscular injection and the adrenal cortical extract is given subcutaneously in divided doses throughout the day.

DISCUSSION

It appears that in its effect on patients with Addison's disease, desoxy-corticosterone acetate treatment simulates qualitatively the action of adrenal cortical extracts. These observations confirm those of Levy-Simpson (26). The potency of desoxy-corticosterone acetate, the constancy and uniformity of the preparation, and the prolonged period of action following a single injection contribute greatly to the success which has attended its use.

The increase in body weight and the elevation of blood pressure following continued treatment with this compound have been uniformly consistent and striking. Another pronounced effect of treatment is a rapid restoration of plasma volume to normal. Determinations of the sodium and chloride concentration of the plasma demonstrate

that frequently the concentration of these ions does not correlate with the clinical condition of the patient. It appears that in all patients adequately treated one may expect a restoration of the plasma concentration of electrolytes to normal. However, during the development of the signs and symptoms of adrenal insufficiency and during the early period of treatment, one may observe little or no correlation between the plasma concentration of sodium and chloride and the clinical state of the patient, but the plasma volume and the total plasma content of sodium and chloride follow closely the changes observed in the condition of the patient.

Because of the very marked sodium and chloride retaining effect which follows treatment with desoxy-corticosterone acetate it is possible to produce edema in patients given an excess of hormone and sodium chloride therapy. Reduction of either the sodium chloride intake or the quantity of desoxy-corticosterone acetate results in a prompt diuresis and a disappearance of edema.

Patients with Addison's disease in whom active tuberculosis exists (JZ and AS) present an added problem. Because of the marked improvement in the general clinical condition of these patients as a result of treatment with desoxy-corticosterone acetate, an attempt is being made to improve the tuberculous lesion by means of sanatorium care.

The close chemical relationship which exists between desoxy-corticosterone (21-hydroxyprogesterone) and progesterone suggests that a sex hormone effect might possibly be induced by injections of large quantities of desoxy-corticosterone acetate. Thus far, however, changes in the breasts or in the menstrual cycle have not been observed in 3 female patients treated with daily injections (2 to 20 mgm daily) of desoxy-corticosterone acetate. It is of interest that in earlier studies it was shown (26) that injections of crystalline progesterone exerted an effect in normal and adrenalectomized dogs which was qualitatively similar to that which occurred following injections of adrenal cortical extracts. The marked "cortin-like" effect which desoxy-corticosterone (21-hydroxyprogesterone) possesses indicates again the close relationship which exists between the hormones of the adrenal cortex and the corpus luteum.

It can not be stated from these studies that

⁴ The authors wish to acknowledge the generous supply of Wilson adrenal cortical extract which was provided for this study by Doctor David Klein, Wilson Laboratories, Chicago, Illinois.

desoxy-corticosterone acetate exerts any specific effect upon carbohydrate metabolism. If the disorder of carbohydrate metabolism in adrenal insufficiency is dependent upon altered sodium, chloride, and potassium metabolism (28) one might expect the disorder to be corrected by desoxy-corticosterone acetate therapy since sodium chloride, and potassium metabolism are apparently restored to normal. In patients in whom adrenal medullary destruction accompanies cortical deficiency one would not expect desoxy-corticosterone acetate treatment to alter any change in carbohydrate metabolism which resulted from a deficit of epinephrine secretion. Until more definite information is obtained, it seems highly desirable to include in the treatment of all patients with Addison's disease a diet high in readily available carbohydrate. There appears to be no indication for reducing the potassium content of the diet of patients adequately treated with desoxy-corticosterone acetate.

One of the remarkable changes which successful treatment introduces is the potentiality of having patients develop rather acutely the signs of adrenal insufficiency when therapy is discontinued suddenly even for as short a period as 72 hours. This effect has also been observed in patients following the sudden withdrawal of adequate quantities of adrenal cortical extract or sodium chloride therapy. The sudden withdrawal of adequate treatment results in a marked diuresis, an increase in the renal excretion of sodium and chloride, potassium retention, a decrease in plasma volume, and a decrease in the total plasma content of sodium and chloride. Within 72 hours after the withdrawal of treatment, signs and symptoms of acute adrenal insufficiency may appear. In this respect an adequately treated patient responds to the sudden withdrawal of treatment in a manner very similar to the response of normal animals to bilateral adrenalectomy. Although experimental evidence (29) indicates that atrophy of the adrenal cortex may follow intensive hormone therapy in normal animals it is not likely that this is of clinical significance since well developed symptoms of Addison's disease rarely occur until extensive destruction of adrenal cortical tissue is present. At present it would seem entirely unjustifiable to withhold substitution therapy from a patient with Addison's disease, in whom the administration of

sodium salts or a diet of low potassium content did not permit the resumption of normal activity.

Although the effect of treatment with this hormone has been uniformly striking in all of the patients, it is realized that the period of treatment in many instances is too short to do other than demonstrate the nature of the changes produced. In all of the patients, the effect of desoxy-corticosterone acetate treatment was observed during at least 1 period in which no sodium chloride therapy was added, and in 5 patients (E V, C N, A S F M and J S) an attempt is being made to maintain these patients over long periods of time with no treatment other than desoxy-corticosterone acetate alone.

SUMMARY

Intramuscular injections of a solution of synthetically prepared desoxy-corticosterone acetate in oil (2 to 30 mgm. daily) resulted in marked improvement in the clinical condition of 8 patients with Addison's disease during a period in which *added sodium chloride therapy* was withheld. The changes associated with desoxy-corticosterone acetate treatment were (a) increase in body weight, (b) elevation of blood pressure, (c) increase in plasma volume, (d) restoration of plasma concentration of sodium, chloride and potassium to normal levels (e) positive sodium and chloride balance, (f) increased renal excretion of potassium and inorganic phosphorus, (g) improved muscular strength and sense of well being.

Withdrawal of desoxy-corticosterone acetate treatment (48 to 72 hours) resulted in (a) weight loss (b) decrease in plasma volume, (c) decrease in total plasma content of sodium and chloride, (d) a negative sodium and chloride balance (e) retention of potassium and inorganic phosphorus, (f) muscular weakness, (g) loss of appetite, and (h) the appearance of symptoms of adrenal insufficiency. Resumption of treatment again resulted in marked improvement and in the specific changes noted above.

CONCLUSIONS

Intramuscular injections of synthetically prepared desoxy-corticosterone acetate appear to reproduce in patients with Addison's disease all of the known effects of treatment with potent adrenal cortical extracts. It is possible to maintain

patients with Addison's disease in excellent condition by means of this treatment without added sodium chloride therapy

The authors are indebted to Miss Mildred Caldwell, Supervisor of the Metabolism Ward, Miss Elizabeth Olsen, Dietician-in-charge of the Metabolism Ward, Mrs Florence White of the Biochemical Laboratory of the Johns Hopkins Hospital, Harry Eisenberg, Kay Eisenberg, and William Sause of the Chemical Division of the Department of Medicine, Johns Hopkins Medical School for their technical assistance and continued cooperation

PROTOCOLS OF PATIENTS

J Z (Number 155645), a 19-year-old Italian boy, was admitted to the Johns Hopkins Hospital on October 27, 1938 with a diagnosis of Addison's disease. His illness began in the spring of 1936 with the gradual onset of loss of strength, appetite and weight, and a slowly progressive generalized brown pigmentation of the skin. In September 1936 he began to have persistent vomiting. At this time a diagnosis of Addison's disease was made and treatment with sodium chloride and adrenal cortical extract^a (40 to 80 grams fresh adrenal cortex) daily, injected subcutaneously. On several occasions during the past 2 years he has required intravenous saline, and has continued to be markedly underweight. In 1937 tubercle bacilli were found in his urine. During his illness the patient had lost 25 pounds in weight.

In 1928 the patient's brother died of tuberculosis and in 1930 his mother died of the same disease. Another brother and sister were sent to a preventorium in 1933 as tuberculosis suspects, although the disease was never proved in these siblings.

Physical examination on admission, October 27, 1938, revealed a thin, poorly nourished and underdeveloped white boy, appearing weak and tired. His temperature was 99.4° F, pulse rate 88 per minute, respirations 18 per minute, and blood pressure 90/50 mm Hg. The skin was dry and pale, and there was generalized diffuse brownish pigmentation over the neck, trunk and extremities becoming almost black over the knees, thighs, and gluteal folds. Small cervical and axillary lymph nodes were palpable but there was no general glandular enlargement. The lungs were clear and the heart normal.

The laboratory data on admission were as follows: hemoglobin 48 per cent (115 grams), red blood cells, 3.69 million, hematocrit 34 per cent cells, white blood cells 4,760 with polymorphonuclears 68 per cent, lymphocytes 22 per cent, monocytes 10 per cent. The sedimentation rate was 25 mm in 1 hour, corrected. The blood Wassermann was negative. The blood nonprotein nitrogen was 40 mgm per cent and blood sugar 70 mgm per cent. The serum CO₂ combining power was 43.8 volumes per cent, the serum concentration of sodium 131.2 meq,

^a Wilson and Company adrenal cortical extract.

chloride 91.8 meq, and potassium 6.08 meq per liter. The total serum protein was 7.2 grams.

The urine was found to have a specific gravity of 1.020 and contained a trace of albumin and many leukocytes but no red cells or casts. Acid fast bacilli were obtained from urine specimens both by culture and guinea pig inoculation.

An x-ray of the chest showed inactive fibroid infiltration in the left apex. In a flat plate of the abdomen, calcium shadows were visible in both suprarenal regions, and stereoscopic x-rays of the skull showed numerous calcium shadows scattered throughout the brain which were interpreted as being healed tubercles.

The basal metabolic rate was plus 18 per cent. On December 17, a phenolsulphonthalein test showed a dye excretion of 30 per cent in 30 minutes and 52 per cent at the end of 2 hours. An electrocardiogram gave a normal tracing.

Résumé of treatment with desoxy-corticosterone acetate. Because of the presence of an active tuberculous lesion of the urinary tract and the very poor clinical condition of the patient 15 grams of added sodium chloride were continued during the institution of desoxy-corticosterone acetate treatment (20 to 30 mgm, daily). This treatment resulted in marked improvement in the patient's general condition, increased appetite and sense of well-being, marked increase in weight, and increased blood pressure. Two weeks after desoxy-corticosterone acetate treatment had been instituted, the added sodium chloride was entirely discontinued, the patient's progress being maintained successfully by means of desoxy-corticosterone acetate treatment alone. Subsequently, withdrawal of desoxy-corticosterone acetate treatment resulted in a rapid onset of the symptoms of adrenal insufficiency. At this time resumption of desoxy-corticosterone acetate treatment without added sodium chloride resulted in marked improvement in the patient's clinical condition. (Data are given in the corresponding charts and tables of the text.)

After being maintained successfully for 20 days by means of desoxy-corticosterone acetate treatment alone (25 to 30 mgm daily) an attempt was made to determine the requirement of hormone when sodium chloride treatment was added (sodium chloride therapy alone had never been sufficient to maintain this patient). It was found that a single daily injection of 5 mgm of desoxy-corticosterone acetate daily was sufficient for this patient when 15 grams of sodium chloride were added to his diet. At present he is being treated successfully by this means and an attempt is being made to treat the active tuberculous lesion in the genito-urinary tract.

A S (Number 151599), a 32-year-old white male bookkeeper, was admitted to the Johns Hopkins Hospital September 12, 1938, complaining of weakness, nausea, and vomiting. The patient dated his present illness from October 1935 when he fractured 3 lumbar vertebrae as the result of a fall. He was hospitalized for 6 weeks at that time and never regained his strength completely. In December 1935, his right wrist became swollen and

painful and has continued to be stiff ever since. In January 1936 he had an attack of pleurisy with pain in the right chest, night sweats and fever lasting 11 days. Thereafter he continued to feel weak, he tired easily and his appetite was markedly decreased. In July 1936 he developed epididymitis on the right side which required incision and drainage. A draining sinus has persisted ever since. Six months before admission he began to lose weight. Weakness and exertional dyspnea became more marked and he noticed dizziness on bending over and had frequent attacks of nausea and vomiting. During this period a progressive deepening in the color of the skin of his face, neck, and arms became apparent. He lost 13.6 kgm. in the course of his illness.

Physical examination on September 12, 1938, revealed a thin man, appearing tired and weak. His temperature was 99.8 F., pulse 96 per minute, respirations 16 per minute and blood pressure 100/64 mm. Hg. There was diffuse light brownish pigmentation of the skin of the face, neck, arms, hands, nipples, and genitalia with splotchy pigmentation over the back and hips. Some pigmentation was noted just inside the lip margins. Coarse bronchial rales were heard scattered throughout both lungs but there was no change in resonance or breath sounds. The heart was normal and the abdomen negative. The right epididymis was enlarged, firm and slightly tender and 2 scars were present on the scrotum from both of which drained a small amount of serous discharge. The prostate was moderately enlarged, tender and undurated. The right wrist was stiff and painful on motion or pressure.

The laboratory data on admission were as follows: The red blood cells were 4.96 million, hemoglobin 94 per cent (13.7 grams), white blood cells 10,320 with 51 per cent polymorphonuclears, 39 per cent lymphocytes, 3 per cent monocytes, 6 per cent eosinophils and 1 per cent basophils. The volume of packed red cells was 43 per cent. The blood Wassermann was negative. The blood nonprotein nitrogen was 40 mgm. per cent, blood sugar 75 mgm. per cent, plasma CO₂ combining power 39.3 volumes per cent, and total plasma protein 7.06 grams per cent. The serum concentration of sodium was 122.5 meq. per liter and of chloride 88.6 meq. per liter.

The urine examination revealed a specific gravity of 1.010, a trace of albumin and no sugar. Microscopic examination showed 3 to 5 red blood cells and 50 to 60 white blood cells per high power field and an occasional hyaline cast. Acid fast organisms were recovered from the urine on culture and guinea pig inoculation. An electrocardiographic tracing was normal.

X rays revealed fibroid infiltration of both apices due to quiescent tuberculosis, no adrenal calcification and a destructive arthritis of the right wrist. The basal metabolic rate was +1 per cent. A phenolsulphonphthalein test showed 25 per cent excretion of the dye in one-half hour and 50 per cent in 2 hours. The tuberculin test was negative in dilutions up to 1:10,000.

In an attempt to establish the diagnosis of Addison's disease the patient was placed on a constant diet and

after a 3-day control period the sodium and chloride balance was studied during the injection of adrenal cortical extract (8 cc. which contained the equivalent of 400 grams of fresh gland) administered daily for 3 days in divided doses subcutaneously. During the period on extract there was a definite retention of sodium and chloride which was followed by a marked loss when extract was discontinued.

The patient was then placed on a high sodium chloride intake and 2 cc. of adrenal cortical extract* (100 grams of fresh gland) were administered subcutaneously each day. On this regimen he gained weight, his appetite increased, nausea disappeared, and the blood electrolytes returned to normal values.

It was felt that the diagnosis of Addison's disease was definite and that, in addition, the patient had tuberculosis of the prostate and epididymis and an old tuberculous arthritis of the right wrist. On November 15, 1938, this patient was readmitted for trial of desoxy-corticosterone acetate therapy.

Resumé of treatment with desoxy-corticosterone acetate. Blood examination on admission revealed the following values: serum sodium 139.3 meq., serum chloride 99.4 meq. and serum potassium 7.08 meq. per liter, carbon dioxide combining power 54.1 volumes per cent, nonprotein nitrogen 31 mgm. per cent, sugar 78 mgm. per cent, hematocrit 40.0 per cent cells, and total protein 6.68 grams per cent. Desoxy-corticosterone acetate treatment (20 mgm. daily) was substituted for the combined sodium chloride and extract treatment. The patient felt well, gained weight, and showed a striking gain in plasma volume. Withdrawal of desoxy-corticosterone acetate treatment resulted in weight loss, decreased appetite, hemoconcentration, a lowering of the serum concentration of sodium and chloride, a marked diuresis, and increased renal excretion of sodium and chloride. Again, treatment with desoxy-corticosterone acetate (20 mgm. daily) without added sodium chloride or extract therapy resulted in marked gain in weight and improved clinical status. (Data are given in the corresponding charts and tables in the text.) At present this patient has been maintained in good condition for more than 4 months by means of a single daily injection of 10 mgm. of desoxy-corticosterone acetate without added sodium chloride therapy. An attempt is now being made to treat this patient's urogenital tuberculosis since his general condition otherwise is compatible with his resuming work as a bookkeeper.

F. M. (Number 144800) a 32-year-old truck driver was first admitted to the Johns Hopkins Hospital on August 13, 1938, complaining of weakness, nausea, and pigmentation of the skin. His present illness began in March 1936 2½ years previously when, following a severe sore throat, he began to notice dizziness, weakness, and nausea. He had an attack of persistent vomiting lasting a week and was told by his doctor that his blood pressure was low. For 3 months he was unable to work but then improved sufficiently to return to his duties as

*Wilson and Company adrenal cortical

truck driver. However, he never fully regained his strength and continued to have attacks of nausea and vomiting at irregular intervals lasting 7 to 10 days. During his illness he lost 68 kgm. in weight. In the summer of 1936 he was troubled with cramps in his legs and some diarrhea. During the 6 months prior to admission he noticed increased brown pigmentation of his face, neck, and forearms.

Physical examination on admission, August 13, 1938, revealed a well developed, fairly well nourished white male appearing rather quiet and listless but not acutely ill. The temperature was 99.8° F, pulse rate 64 per minute, respirations 20 per minute, blood pressure 90/60 mm Hg, and weight 64.1 kgm. There was generalized brownish pigmentation, especially of exposed parts, perineal region, and scars. There were several dark nevi on the face and a small pigmented area on the buccal mucosa. The heart and lungs were normal.

The laboratory data revealed the following: Red blood cells 3.55 million, hemoglobin 73 per cent (107 grams), white blood cells 5,440, polymorphonuclears 42 per cent, lymphocytes 52 per cent, eosinophils 2 per cent, basophils 4 per cent, volume of packed red blood cells 32 per cent. The blood Wassermann reaction was negative. The blood nonprotein nitrogen was 44 mgm per cent, blood sugar 77 mgm per cent, serum concentration of sodium 132.1 m.eq., and of chloride 98.4 m.eq. per liter, plasma carbon dioxide combining power 45.8 volumes per cent, total protein 6.6 grams per cent, serum cholesterol 244 mgm per cent, serum calcium 11.2 mgm per cent, and phosphorus 4.9 mgm per cent.

The urine showed a specific gravity of 1.020. No albumin or sugar was present. On microscopic examination occasional white blood cells and hyaline casts were observed.

The phenolsulphonthalein test showed an excretion of 15 per cent in 30 minutes and 65 per cent in 2 hours. The basal metabolic rate varied between -12 and -23 per cent. X-rays of the chest were clear, no calcification of the adrenals was noted, the sinuses showed clouding of the left antrum. The tuberculin test was positive (1:1,000).

Course Following admission the patient was provided with a constant diet to which was added 15 grams of sodium chloride. The patient showed a positive sodium and chloride balance during a 3-day period in which 4 cc. of adrenal cortical extract were injected twice daily. A negative sodium and chloride balance was noted preceding and following the period of treatment. Following these observations a high sodium chloride intake was maintained and the patient showed a steady gain in weight and improvement in appetite during his 3 weeks in the hospital. At the time of discharge, September 3, 1938, the serum concentration of sodium was 139.9 m.eq. and chloride 102.0 m.eq. per liter. The blood nonprotein nitrogen was 32 mgm per cent, plasma carbon dioxide combining power 56.9 volumes per cent, blood pressure 100/70 mm. Hg, and body weight 65.9 kgm.

Following discharge from hospital he continued to take

15 grams of sodium chloride daily and felt stronger than before admission but he was still unable to carry on his work as truck driver. On October 18, 1938, he was readmitted for trial of desoxy-corticosterone acetate therapy.

Résumé of treatment with desoxy-corticosterone acetate Blood examination on admission revealed the following values: serum sodium 136.0 m.eq., serum chloride 96.6 m.eq., and serum potassium 6.72 m.eq. per liter, carbon dioxide combining power 47.5 volumes per cent, nonprotein nitrogen 38 mgm. per cent, sugar 73 mgm. per cent, hematocrit 46.3 per cent cells, and total protein 6.53 grams per cent. Desoxy-corticosterone acetate treatment (15 mgm daily for 9 days) resulted in marked improvement in his muscular strength, appetite, and sense of well-being. Withdrawal of desoxy-corticosterone acetate treatment (3 days) resulted in weight loss, some loss of strength and appetite, a marked increase in the renal excretion of sodium, and a rise in the serum concentration of potassium. Resumption of desoxy-corticosterone acetate treatment at this time resulted in marked improvement accompanied by a gain in weight and strength and an increase in blood pressure. (Data are given in the corresponding charts and tables of the text.) At no time during these studies was added sodium chloride given. At present, this patient is working at his occupation as truck driver, his treatment consisting of 10 mgm of desoxy-corticosterone acetate daily without added sodium chloride therapy.

C N (Number 100114), a married, female telephone operator, 34 years of age, was admitted to the Johns Hopkins Hospital on June 3, 1936, complaining of weakness, weight loss, nausea, and vomiting. Three years before admission the patient had noticed increasing fatigue and some darkening of her skin. For two weeks before admission nausea had been present almost constantly and the patient had been confined to bed. During her illness she had lost 15.9 kgm in weight. Until the present illness her menses had been normal. For one year prior to admission her menses had been irregular. In 1932 and again in 1933 she had had a peritonsillar abscess.

Physical examination on admission June 2, 1936, revealed a pale woman who appeared to be rather weak and showed evidence of considerable weight loss. Her temperature was 97.2° F, pulse rate 82, respirations 20, and blood pressure 86/60 mm Hg. Her skin was slightly tanned. There was dark pigmentation of the borders of the lips and a large blotchy brown area over the lower left abdomen. The heart sounds were distant and of poor quality, and the radial pulses were feeble. The physical examination was otherwise normal.

The laboratory findings were as follows: Hemoglobin 106 per cent, red blood cells 5.3 million (hemoconcentration), white blood cells 12,750, polymorphonuclears 64 per cent, lymphocytes 26 per cent, monocytes 8 per cent, eosinophils 2 per cent, basophils 1 per cent, cell volume 48 per cent, blood Wassermann test negative, nonprotein nitrogen 46 mgm. per cent, sugar 80 mgm per cent, plasma sodium concentration 138.9 m.eq. per liter, and

the basal metabolic rate —11 per cent. The urine was normal.

A trial of salt deprivation was begun, and in 3 days a typical Addisonian crisis occurred. Plasma sodium concentration fell from a level of 138.9 mEq to 124 mEq. per liter and the patient became semicomatose, the blood pressure being 66/44 mm. Hg. Rapid recovery followed the administration of cortical extract and the intravenous infusion of sodium chloride and glucose. The patient was discharged on June 20 1936, feeling somewhat weak but otherwise improved.

During the next year the patient continued to be in fair health although she was never strong enough to do her housework. On August 28 1937 she was admitted to the hospital in a severe crisis. Following large amounts of salt and glucose by vein and extract by vein and subcutaneously she improved rapidly and remained well on injected extract and a high salt (10 to 12 grams of NaCl daily) and carbohydrate intake.

Since September 18, 1937 orally administered* extract had been substituted for injections of extract. Her health continued to improve, and 5 months later she was able to return to her former work on a part time basis in addition to doing her own housework and gardening. On October 17 1938 she was readmitted for trial of desoxy corticosterone acetate therapy.

Résumé of treatment with desoxy-corticosterone acetate
On admission, blood examination revealed the following values: serum sodium 131.4 mEq., serum chloride 95.0 mEq. and serum potassium 5.00 mEq. per liter; carbon dioxide combining power 56.0 volumes per cent, non protein nitrogen 40 mgm. per cent, sugar 78 mgm. per cent, and total protein 6.88 grams per cent. Desoxy corticosterone acetate (10 to 15 mgm. daily) was substituted for the orally administered extract, the sodium chloride therapy being continued. The substitution of desoxy-corticosterone acetate treatment (sodium chloride 15 grams daily being continued) resulted in marked clinical improvement, an increase in blood pressure, a very marked retention of sodium and chloride and a striking gain in weight. On the 7th day of treatment as a result of the marked retention of sodium, moderate edema of the face and extremities was apparent. Withdrawal of desoxy-corticosterone acetate treatment (sodium chloride 15 grams daily being continued) resulted in a marked diuresis, marked weight loss, and after 72 hours in loss of appetite and strength. Resumption of desoxy corticosterone acetate treatment (5 to 10 mgm. daily) resulted in prompt improvement and again was accompanied by a marked gain in weight. At this time the added sodium chloride therapy (15 grams daily) was entirely discontinued and the patient was treated with desoxy-corticosterone acetate (5 mgm. daily) only (Data are given in the corresponding charts and tables of the text.)

This patient has now been maintained in good condition for 5 months by means of desoxy-corticosterone

acetate treatment (2 to 5 mgm. daily) without added sodium chloride therapy. On this regimen she has been capable of carrying on her duties as housewife. Furthermore, the patient states that she feels better now than she has in several years.

J. S. (Number 155073), a 29-year-old telegraph clerk, was admitted to the Johns Hopkins Hospital on October 21 1938, complaining of nausea, vomiting, and gaseous distention. The present illness began in 1934 with gradual onset of abdominal distention and discomfort chiefly after meals. At the same time dizziness on arising was noted. In 1936 he first noticed some brownish pigmentation on his nose and upper lip which increased only slightly during the subsequent two years. During his illness his weight decreased from 72.7 kgm. to 56.8 kgm. In 1936 he was told his blood pressure was 90 mm. Hg systolic, but during the year prior to admission it was found to be 110 mm. Hg.

Physical examination on October 21 1938, revealed a tall, thin, anemic white male, slow of speech and movement, and appearing weak and emaciated, but not acutely ill. The temperature was 99 F., pulse rate 88 per minute, respirations 22 per minute, and blood pressure 115/70 mm. Hg with the patient in a recumbent position, 90/60 mm. Hg with the patient in an upright position. There was widespread brownish pigmentation, particularly of the finger joints, genitalia, and buttocks. There were several dark nevi on the thorax. The lungs were clear and the heart sounds were normal.

The laboratory data were as follows: Hemoglobin was 104 per cent (151 grams) red blood cells 5.19 million, white blood cells 5,350 with polymorphonuclears 71 per cent, lymphocytes 24 per cent, monocytes 4 per cent, and eosinophils 1 per cent. The hematocrit was 47 per cent cells. The blood Wasserman was negative. The blood nonprotein nitrogen was 32 mgm. per cent and the blood sugar 84 mgm. per cent. The serum concentration of sodium was 138.5 mEq., chloride 96.4 mEq., and potassium 6.48 mEq. per liter. The urine and stool were normal.

X rays of the heart and lungs were normal and examination of the gastro-intestinal tract gave no evidence of any organic lesion. No adrenal calcification was noted. The tuberculin test was negative in dilutions up to 1:1000. An electrocardiogram showed a normal record.

Résumé of treatment with desoxy-corticosterone acetate
This patient had been receiving previously 5 grams of added sodium chloride daily and 2 cc. of adrenal cortical extract* injected subcutaneously. This treatment was discontinued and desoxy-corticosterone acetate treatment (15 mgm. daily) resulted in marked clinical improvement, associated with increased appetite, improved muscular strength, increased weight, and increased blood pressure. Withdrawal of desoxy-corticosterone acetate treatment (48 hours) resulted in weight loss, lowering of blood pressure, anorexia, diuresis, and a marked increase in the renal excretion of sodium and chloride. Resumption of desoxy-corticosterone acetate treatment (20 mgm.

* Wilson and Company adrenal cortical extract added to glycerol.

* Parke, Davis and Company Eschatin.

daily) resulted in striking improvement in the clinical condition of the patient, gain in weight, improved appetite, and strength. (Data are given in the corresponding charts and tables of the text.) At no time during this study was added sodium chloride administered. At present this patient is being maintained on 10 mgm. of desoxy-corticosterone acetate daily without added sodium chloride therapy.

E V (Number 116221), a Greek male store clerk, 39 years of age, was admitted to the Johns Hopkins Hospital, August 10, 1937, complaining of abdominal pain, progressive weakness, and darkening of the skin. During the 2 years prior to his present admission, his skin had become darker and he had had recurrent attacks of nausea and vomiting with slowly progressive weakness. During this present illness he had lost 127 kgm.

On admission, the patient was found to be very weak, and there was evidence of marked weight loss. His temperature was 99° F, pulse rate 88, respirations 24, and body weight 519 kgm. His skin was uniformly dark brown, with an increase in the degree of pigmentation in the folds of the axillae and palms. There was a diffuse black speckled pigmentation on the buccal mucous membrane. The blood pressure was 78/44 mm. Hg and the heart sounds were very distant.

Laboratory examination on admission revealed the following: Hemoglobin 74 per cent, red blood cells 4.34 million, white blood cells 3,880, polymorphonuclears 59 per cent, lymphocytes 32 per cent, monocytes 4 per cent, eosinophils 1 per cent, basophils 4 per cent. The urine was normal. The blood Wassermann was negative. The fasting blood sugar was 90 mgm. per cent, the blood nonprotein nitrogen 60 mgm. per cent, and the plasma concentration of sodium 130.7 meq, chloride 97.4 meq, and potassium 4 meq per liter. X-rays of the chest and abdomen were negative. A tuberculin test was positive 0.001 mgm.)

On a high sodium, low potassium diet, the patient gained strength and weight, and the plasma electrolytes returned to a normal concentration in 1 week. The patient was discharged August 25, 1937, weighing 53.7 kgm., with instructions to take 12 grams of sodium chloride daily. He continued in fair health although his appetite was poor, and he never felt strong enough to return to work. Since December 1937 the patient has received 4 cc. (160 grams of adrenal cortex) daily of injected extract⁹ and 12 grams of sodium chloride. On October 5, 1938, the patient was readmitted for desoxy-corticosterone acetate therapy.

Résumé of treatment with desoxy-corticosterone acetate Blood examination on admission revealed the following values: serum sodium 137.5 meq., serum chloride 103.0 meq, and serum potassium 5.40 meq per liter, carbon dioxide combining power of the serum 53.2 volumes per cent, nonprotein nitrogen 32 mgm per cent, blood sugar 79 mgm. per cent, hematocrit 35.8 per cent cells, and total protein 5.90 grams per cent. Withdrawal of sodium chloride and extract treatment (24 hours)

resulted in loss of strength, hemoconcentration (the cell volume increasing from 36.0 per cent cells to 39.3 per cent cells), a marked decrease in plasma volume (from 2,560 cc. to 2,380 cc.), a marked diuresis associated with a negative sodium and chloride balance, a striking decrease in the sodium and chloride concentration of the serum, and a slight rise in the serum concentration of potassium. At this time the injection of 15 mgm. of desoxy-corticosterone acetate daily, without added sodium chloride therapy, resulted in marked clinical improvement. On 2 occasions withdrawal of desoxy-corticosterone acetate treatment (48 hours) resulted in marked weight loss, anorexia, and decrease in strength. In both instances resumption of treatment caused the prompt disappearance of these signs and symptoms. (Data are given in the corresponding charts and tables of the text.)

This patient has been treated successfully for 5 months by means of daily injections (10 to 15 mgm. per day) of desoxy-corticosterone acetate. Throughout this entire period the patient has had a normal diet with no added sodium chloride therapy. At present he is being maintained in excellent condition on a single daily injection of 10 mgm of desoxy-corticosterone acetate without added sodium chloride. On this regimen he has returned to work for the first time in over 3 years.

P W (Number 150453), a 34-year-old clergyman, was admitted to the Johns Hopkins Hospital on October 5th, 1938, for treatment of Addison's disease. Generalized brownish pigmentation was first noticed in 1925. In the summer of 1932-33 he had heat cramps which were relieved by salt water. In 1934 he had an attack of nausea, vomiting, and weakness progressing to coma. At this time he was found to have a blood pressure of 70/50 mm. Hg. Treatment with sodium chloride, glucose, and adrenal cortical extract resulted in marked improvement, and since then he has been maintained in fair health on small doses of cortical extract¹⁰ (2 to 4 cc. daily representing approximately 80 to 160 grams of fresh cortex) and 8 to 10 grams of added sodium chloride daily. On 2 occasions he has been on the verge of collapse necessitating intravenous therapy.

In 1930 the patient had a severe sore throat, in 1932 a tonsillectomy was performed, and in 1934 he underwent an appendectomy.

Physical examination on October 5, 1938, revealed a white male of slight build, fairly well nourished, appearing alert, and in no discomfort. The temperature was 98.2° F, pulse rate 84 per minute, respirations 22 per minute, and blood pressure 94/60 mm Hg. The skin was slaty brown in color with dark brown pigmentation of the face, neck, forearms, external genitalia, and gluteal folds. Over the face, neck, and shoulders were a few scattered deeply pigmented nevi. No pigmentation of the mucous membranes was observed. The external ears were hard and cartilaginous. The lungs were clear, and the heart was normal save for the heart sounds being distant.

Laboratory data on admission was as follows. The

⁹ Wilson and Company adrenal cortical extract

¹⁰ Wilson and Company adrenal cortical extract.

red blood cells were 4.3 million, hemoglobin 12.7 grams white blood cells 5,000 with polymorphonuclears 69 per cent, lymphocytes 25 per cent and monocytes 6 per cent. The sedimentation rate was 12 mm. in 1 hour and the volume of packed red blood cells 43 per cent. The blood Wassermann was negative. The blood nonprotein nitrogen was 38 mgm. per cent, blood sugar 65 mgm per cent, plasma CO₂ combining power 57.9 volumes per cent, serum concentration of sodium 130.5 meq, chloride 97.8 meq and potassium 5.16 meq per liter and total plasma protein 7.38 grams per cent. Examinations of the urine was negative.

The basal metabolic rate was plus 4 per cent. X ray of the heart and lungs was normal. There was no evidence of calcification in the area of the adrenals. An electrocardiographic tracing showed T1 isoelectric and T2 and T3 inverted.

Résumé of treatment with desoxy-corticosterone acetate Withdrawal of sodium chloride and extract treatment (48 hours) resulted in the onset of symptoms of acute adrenal insufficiency associated with a marked lowering of blood pressure, anorexia, vomiting a diuresis associated with an increased excretion of sodium and chloride, a further lowering of the serum concentration of sodium and chloride and a slight rise in the serum concentration of potassium. At this time 15 mgm. of desoxy-corticosterone acetate were injected and the patient was given a single infusion of 16 grams of sodium chloride (a quantity of sodium chloride calculated to be equivalent to the negative balance of sodium chloride which had obtained during the 2 days off treatment). Subsequently the patient received 15 to 20 mgm. of desoxy-corticosterone acetate daily no additional sodium chloride being given. The patient was maintained on this regimen for 23 days with marked clinical improvement, increased appetite, gain in body weight, and increased blood pressure. (Data are given in the corresponding charts and tables of the text.)

At this time it was decided to test the dose of desoxy corticosterone acetate which would be required when sodium chloride therapy (15 grams daily) was added to the regimen. Previous experience had shown that this patient could not be maintained on sodium chloride therapy alone. Following the addition of 15 grams of sodium chloride to the diet, it was observed that approximately 5 to 6 mgm. daily of desoxy-corticosterone acetate were sufficient to maintain him in excellent condition.

J P (Number 123218) a 40-year-old Italian housewife was admitted to the Johns Hopkins Hospital on October 21 1937 complaining of weakness, weight loss, and pigmentation of the skin of 3 years duration. The present illness began in 1934 when the patient first began to notice brownish black freckles on her forearms. This pigmentation gradually became deeper and spread until her whole body had become a chocolate brown. During the year preceding admission she had noticed increasing fatigue, weakness, and anorexia, her weight decreasing from 104.5 kgm. to 66.3 kgm. Menses had always been normal. She had had one normal pregnancy in 1920 and one miscarriage 5 months later

Physical examination on admission revealed a woman appearing moderately weak and showing evidence of marked loss of weight. The temperature was 98.2 F pulse rate 72, respirations 18 and blood pressure 133/95 mm Hg. There was marked dark brown pigmentation of the whole body with accentuation in the flexor creases but with a striking absence of pigmentation of the palms and soles. There was a blotchy pigmentation of the lips and buccal mucous membranes. There was very mild narrowing of the retinal arteries. The heart sounds were rather distant, and a soft systolic murmur was heard at both base and apex. The peripheral vessels were soft.

The laboratory findings were as follows Hemoglobin 13.4 grams red blood cells 4.2 million white blood cells 4,000 polymorphonuclears 60 per cent, lymphocytes 36 per cent, monocytes 3 per cent, eosinophils 1 per cent, hematocrit 40 per cent cells. Specific gravity of the urine was 1.019 and the urine contained 2 plus albumin. The blood Wassermann showed negative findings and the blood chemistry examination revealed blood sugar 74 mgm. per cent, nonprotein nitrogen 28 mgm. per cent, plasma CO₂ 52.2 volumes per cent, total plasma protein 6.44 grams per cent, plasma concentration of sodium 138.6 meq, and chloride 100.0 meq per liter. X rays showed some non tuberculous infiltration of the lungs, a normal heart and aorta, and no intra abdominal calcification. Skull plates were normal. Electrocardiogram showed low voltage and an inversion of the T waves. Basal metabolic rate was +17 per cent.

During the first week the patient was allowed to eat as she desired without extra salt. She gradually became weaker, appetite failed and she lost weight. On November 2, 1937 the plasma concentration of sodium was 127 meq and of chloride 91 meq the nonprotein nitrogen had risen to 42 mgm. per cent. Hematocrit was still 40 per cent cells. Improvement followed the subcutaneous injection of extract, however and by November 8th the plasma sodium had risen to 138.6 meq and chloride to 99.6 meq.

Since January 1938 this patient received 2 cc. (80 grams of adrenal cortex) of injected extract¹¹ daily in addition to the daily intake of 10 grams of sodium chloride. On a recent examination (May 8 1938) she was found to have a systolic blood pressure of over 150 mm. Hg which confirmed the suspicion that she had some degree of hypertension preceding the onset of adrenal insufficiency. On November 14 1938, she was readmitted for trial of desoxy-corticosterone acetate therapy.

Résumé of treatment with desoxy-corticosterone acetate Blood examination on admission revealed the following values serum sodium 138.7 meq serum chloride 100.6 meq and serum potassium 4.20 meq per liter carbon dioxide combining power 55.1 volumes per cent nonprotein nitrogen 32 mgm. per cent, sugar 83 mgm. per cent, hematocrit 41.3 per cent cells, and total protein 6.3 grams per cent. Withdrawal of sodium chloride and extract treatment (48 hours) resulted in weight loss

¹¹ Wilson and Company adrenal cortical extract.

slight loss of strength, decreased appetite, hemoconcentration, and a marked reduction in the serum concentration of sodium and chloride, associated with a marked negative balance of these electrolytes (The onset of catamenia coincided with the withdrawal of treatment.) At this time treatment with desoxy-corticosterone acetate (20 mgm daily) without added sodium chloride therapy resulted in improved strength, appetite and sense of well-being, weight gain, and an increase in blood pressure. Subsequently, the withdrawal (3 days) of desoxy-corticosterone acetate treatment resulted in a recurrence of the symptoms of adrenal insufficiency, associated with weight loss and a lowered blood pressure. Again treatment with desoxy-corticosterone acetate (20 mgm daily) without added sodium chloride therapy resulted in improved strength and appetite, a marked gain in weight and an increase in blood pressure. (Data are given in the corresponding charts and tables of the text.) Following this study, desoxy-corticosterone acetate treatment was discontinued, and the patient was maintained on 15 grams of sodium chloride daily. On this regimen she is able to carry on all of her household duties.

BIBLIOGRAPHY

- Hartman, F A., MacArthur, C. G., and Hartman, W E., A substance which prolongs the life of adrenalectomized cats. *Proc. Soc. Exper Biol and Med.*, 1927, 25, 69
- Rogoff, J M., and Stewart, G N., The influence of adrenal extracts on the survival period of adrenalectomized dogs. *Science*, 1927, 66, 327
- Pfiffner, J J., and Swingle, W W., The preparation of an adrenal extract of the suprarenal cortex. *Anat. Rec.*, 1929, 44, 225
- Reichstein, T., *Chemie des Cortins und seiner Begleitstoffe, Ergebnisse der Vitamin- und Hormonforschung*. Akademische Verlagsgesellschaft, Leipzig, 1938, 1, 334 (Review)
- Steiger, M., and Reichstein, T., Desoxy-corticosteron (21-oxy-progesteron) aus Δ^3 -3-oxy-ätiol-cholensäure. *Helvet chim acta*, 1937, 20, 1164
- Reichstein, T., and v. Euw, J., Über Bestandteile der Nebennierenrinde. Isolierung der Substanzen Q (Desoxy-Corticosteron) und R sowie weiterer Stoffe. *Helvet chim. acta*, 1938, 21, 1197
- Thorn, G W., Engel, L L., and Eisenberg, H., The effect of corticosterone and related compounds on the renal excretion of electrolytes. *J Exper Med*, 1938, 68, 161
- Thorn, G W., and Eisenberg, H., Studies on desoxy-corticosterone (a synthetic adrenal cortical hormone). *Endocrinology*, July 1939 (in press)
- Loeb, R. F., Effect of sodium chloride in treatment of a patient with Addison's disease. *Proc. Soc. Exper Biol. and Med.*, 1933, 30, 808
- Harrop, G A., Weinstein, A., Soffer, L. J., and Trescher, J H., The diagnosis and treatment of Addison's disease. *J A. M. A.*, 1933, 100, 1850
- Wilder, R N., Kendall, E. C., Snell, A. M., Kepler, E. J., Rynearson, E. H., and Adams, M., Intake of potassium, an important consideration in Addison's disease—a metabolic study. *Arch. Int Med.*, 1937, 59, 367
- Butler, A M., and Tuthill, T., An application of the uranyl zinc acetate method for determination of sodium in biological material. *J Biol. Chem.*, 1931, 93, 171
- Peters, J P., and Van Slyke, D D., *Quantitative Clinical Chemistry Vol. II Methods Chapter XXX Chloride*. Williams and Wilkins Co., Baltimore, 1932, p 829
- Shohl, A. T., and Bennett, H B., A micro method for the determination of potassium as iodoplatinate. *J Biol Chem.*, 1928, 78, 643
- Strauss, M B., The use of thorium nitrate in the rapid ashing of serum and urine. I. Adopted for subsequent potassium determinations. *J Biol Chem.*, 1937, 118, 331
- Van Slyke, D D., and Cullen, G E., Studies of acidosis. I The bicarbonate concentration of the blood plasma, its significance and its determination as a measure of acidosis. *J Biol Chem*, 1917, 30, 288.
- Van Slyke, D D., Studies of acidosis. II. A method for the determination of carbon dioxide and carbonates in solution. *J Biol. Chem.*, 1917, 30, 347
- Van Slyke, D D., and Stadie, W C., The determination of the gases of the blood. *J Biol Chem.*, 1921, 49, 1
- Folin, O., and Wu, H., A system of blood analysis. *J Biol Chem*, 1919, 38, 81
- Gregersen, M I., and Gibson, J G., Jr., Conditions affecting the absorption spectra of vital dyes in plasma. *Am J Physiol*, 1937, 120, 494
- Gibson, J G., Jr., and Evans, W A., Jr., Clinical studies of the blood volume. I Clinical application of a method employing the azo dye "Evans blue" and the spectrophotometer. *J Clin. Invest.*, 1937, 16, 301
- Gibson, J G., Jr., and Evelyn, K. A., Clinical studies of the blood volume. IV Adaptation of the method to the photoelectric microcolorimeter. *J Clin. Invest.*, 1938, 17, 153
- Evelyn, K. A., and Cipriani, A. J., A photoelectric microcolorimeter. *J Biol. Chem.*, 1937, 117, 365
- Fiske, C. H., and Subbarow, Y., The colorimetric determination of phosphorus. *J Biol. Chem.*, 1927, 66, 375
- Thorn, G W., Garbutt, H T., Hitchcock, F A., and Hartman, F A., The effect of cortin on the sodium, potassium, chloride, inorganic phosphorus and total nitrogen balance in normal subjects and in patients with Addison's disease. *Endocrinology*, 1937, 21, 202
- Gibson, J G., Jr., and Evans, Wm A., Jr., Clinical studies of the blood volume. II The relation of

- plasma and total blood volume to venous pressure, blood velocity rate, physical measurements, age and sex in 90 normal humans. *J. Clin. Invest.*, 1937, 16, 317
25. Thorn, G. W., and Kuhlman, D., The effect of desoxy corticosterone on carbohydrate metabolism of adrenalectomized dogs. (To be published.)
26. Levy Simpson, S., The use of synthetic desoxy-corticosterone acetate in Addison's disease. *Lancet*, 1938, 2, 557
27. Thorn, G. W., and Engel, L. L., The effect of sex hormones on the renal excretion of electrolytes. *J. Exper. Med.*, 1938, 68, 299
28. Kendall, E. C., Flock, E. V., Bollman, J. L., and Mann, F. C., The influence of cortin and sodium chloride on carbohydrate and mineral metabolism in adrenalectomized dogs. *J. Biol. Chem.*, 1938, 126, 697
29. Ingle, D. J., The effects of administering large amounts of cortin on the adrenal cortices of normal and hypophysectomized rats. *Am. J. Physiol.*, 1938, 124, 369

PROCEEDINGS OF THE THIRTY-FIRST ANNUAL MEETING OF THE
AMERICAN SOCIETY FOR CLINICAL INVESTIGATION
HELD IN ATLANTIC CITY, N J, MAY 1, 1939

READ BEFORE THE SCIENTIFIC SESSION

PRESIDENTIAL ADDRESS

By T. R. HARRISON

If we assume that a presidential address has any useful function at all it follows that this function is to try to benefit the organization to which the address is delivered. Such a purpose can best be served not by praising the accomplishments of the past, but by considering the dangers of the future. Medical societies, as well as other cultural organizations are like individuals in that they tend to grow to reach maturity to accomplish little or much—as the case may be—and then to decay. What are the causes of this institutional arteriosclerosis? Is it inevitable? Can its progress be delayed, arrested or perhaps prevented entirely? By what means? Realizing that other and wiser physicians may prescribe differently for this important disease, my suggestions are as follows:

Since institutional arteriosclerosis is not limited to societies but tends to affect all cultural organizations, the problem should be approached in its broader aspects. A study of the decline of medical schools in the past suggests that there are two general groups of factors.

(1) *Extrinsic causes.* These include political, economic, and other influences which affect society as a whole and which cause a general cultural decline. The decay of the great medical faculties of Salerno, Montpellier and Bologna can possibly be ascribed to such factors which are also responsible for the present catastrophic decline of the medical centers in certain central European countries. These extrinsic causes are largely beyond our control. We are concerned with them as citizens rather than as physicians.

(2) *Intrinsic causes.* These include unfavorable conditions which develop within medical societies and which are therefore subject to control by the membership. Although there are numerous different conditions—such as nepotism, intolerance and self-satisfaction—which tend to have an unhealthy effect, they can all be traced to one general cause—a failure to select the best possible men.

The importance of exhaustive effort to find the most capable individuals for chiefs of departments is generally recognized. Much attention is likewise paid to choosing the proper persons for the secondary places. However, assistants and instructors are often appointed rather casually and too frequently the quality of agreeableness is emphasized to the neglect of more important capacities. This is entirely illogical. The professors of tomorrow must be chosen from the instructors of today. No man should be appointed to a permanent salaried academic position—be it ever so lowly—unless he already gives promise of becoming in the future material for positions

of the highest rank. If the roots of the academic tree are properly cared for the fruit will take care of itself.

Even when external conditions are favorable, medical culture can not flourish for long with mediocre personnel. When, as during the past ten years and possibly for some decades in the future, the extrinsic causes of cultural decline are already operative, it is important that every precaution be taken against intrinsic decay. The only assurance lies in constant—almost agonizing—effort to choose the good man.

But this is not a simple matter. Some persons, in high positions, looking for an able man to fill a vacancy but lacking the patience of Diogenes and the illumination cast by his lantern, tend to become exhausted by the search. They then choose the next individual they encounter, saying, "God made him, and therefore let him pass for a man." A genius—like Gilman or in the clinical field, Peabody—with an almost supernatural ability to pick the right man for the job is a rarity. Are there any criteria whereby we with lesser gifts can be guided in choosing men? I believe there are.

The customary procedure in filling an academic position is to inquire concerning a prospective candidate from his present and past superiors. Since nearly everyone tries to appear at his best in the eyes of his chief, the professors and associate professors often have a false idea of a man's abilities. Why not inquire of his inferiors also? They see him as he is. The house staff of a teaching hospital can usually "size up" the members of the permanent staff as well and sometimes better than the chief can. No man should be seriously considered for academic advancement unless he has the respect—not only of his superiors but also of his inferiors. The old saying that "Young men think old men are fools, but old men know young men are fools" is only partly true, but in any case, young men usually know which other young men are fools.

Appointments are often made or refused on the basis of a man's school, his religion or his social qualifications. I fail to see what significance these factors have. The happy family idea has been much over-emphasized. Even if one regards harmony as the prime desideratum, it will usually be found that truly unusual men get on well—while lesser men, jealous because they are deficient, tend to quarrel.

Much emphasis is placed on administrative ability, but the term is usually not defined. Some consider that a good administrator is a man with a passion for details. If the word is to be used in this

not be confused with another quality—leadership. We should remember that “the greatest clerks be not the wisest men.” An administrator—as defined above—is often preoccupied with his own system of better doing things which are already being done, a leader is concerned with stimulating other men to do things which otherwise would be left undone. Even first rate administration (as defined here) bears a close resemblance to puttering, first-rate leadership resembles nothing else—it is a unique and all-too-rare quality.

The good man is he who not only furnishes ideas but who when working with men of lower rank than himself does his own share and a little more of the actual labor. He says to his inferiors, “Come on,” not “Go on.”

The attitude of a man toward research, his interest in it, and his energy are just as important as his intelligence. All great investigators seem to have had one quality in common—they have labored while others rested.

We all know individuals with fine minds who accomplish little because they lack drive. Such men are like the cat which, “would eat fish and would not wet her feet.” Critical, creative imagination—the most important quality in research—is not immaculately conceived by the mind alone, it is “by” energy “out of” intellect.

It is of major importance that a candidate should really love investigation. He should realize that research at its worst is

“To loose good dayes, that might be better spent,
To wast long nights in pensive discontent,
To speed to-day, to be put back to-morrow,
To feed on hope, to pine with feare and sorrow
To fret thy soule with crosses and with cares,
To eat thy heart through comfortlesse dispaire,
To fawne, to crouche to waite, to ride to ronne,
To spend, to give, to want, to be undone.”

while at its best, research leads to “infinite riches in a little room.”

The qualities which have been mentioned are only a few of the ones which mark the good man. They have been stressed because they seem to me especially important and because they are often overlooked. Many other qualities might be cited but in a final analysis most of them can be reduced to two traits of transcending importance. The first of these is wisdom. Such wisdom includes a broad knowledge of medicine in general and a deep understanding of certain fields of medicine. Aside from this purely intellectual quality there is an emotional trait which is perhaps even more important. This is a certain radiant energy which, operating internally, keeps the individual constantly working and which, operating externally, catalyses other men to similar action. For want of a better term we may, with apologies for the mixed metaphor, call this quality “contagious fire.”

In a world of crumbling standards the safest assurance for the future of academic medicine lies in the thoughtful selection of the best possible young men. Our choosing should be tempered by the sober recollection that each corporal should with justice carry in his knapsack the baton of a marshal.

Contribution to the Etiology of Diabetic Retinitis by JONAS S. FRIEDENWALD and (by invitation) MANUEL G. GICHNER, Baltimore, Md.

Patients with active hemorrhagic retinitis in diabetes have increased capillary fragility. The capillary fragility is improved, but is not as a rule brought back to normal by large doses of vitamin C. Saturation tests with ascorbic acid reveal a marked deficiency in the ability to excrete this substance in the urine following the injection of large doses by mouth. Absorption from the gastro-intestinal tract is apparently normal as is also the renal threshold. Hence the excretion deficit is to be attributed to abnormal utilization within the body. In a small group of cases the administration of vitamin B complex resulted in a return to normal of the capillary resistance, and also in a reappearance of the ability to excrete injected ascorbic acid.

Vitamin C Nutrition and Metabolism in Rheumatoid Spondylitis JAMES F. RINEHART, San Francisco, Cal.

A detailed study of the nutritional status relative to vitamin C was made in a series of cases of rheumatoid spondylitis. In 32 cases the average fasting blood plasma vitamin C value was 0.12 mgm per 100 cc. In 90 per cent of cases the initial value was below 0.40 mgm per 100 cc. In a number of the cases, determinations of blood plasma ascorbic acid were made following administration of large oral doses of vitamin C (15 mgm per kilogram). The curves were ‘flat’ indicating significant undersaturation of tissues.

Data gained from dietary histories indicate that although some of the cases were ingesting grossly inadequate amounts of vitamin C, this was not uniformly so. Many cases showed depleted vitamin C reserves in spite of a normally adequate dietary intake. In several cases the metabolic fault was striking. Possible factors responsible for this abnormality are considered.

The influence of known supplements of vitamin C upon the blood ascorbic acid sedimentation rate, capillary strength, weight, general condition, and arthritis in a group of cases followed for two months or longer is analyzed.

The capillary strength was determined initially in 27 cases. This was found to be almost uniformly lowered. In 11 of 14 cases followed for two months or more the capillary strength rose after administration of supplementary vitamin C. Eight of 11 cases gained weight. Twelve of 17 showed slowing of the sedimentation rate occurring within 4 months. Improvement in general condition and diminution of pain was almost regularly observed. The only other treatment was physiotherapy in a portion of the cases.

These data indicate that vitamin C deficiency is almost uniformly present in this form of arthritis. This deficiency may occur in the presence of a normally adequate vitamin C intake. The uniform finding of vitamin C depletion and the response to liberal vitamin C supplements noted suggest that the deficiency is contributory to the disease.

The Nature of Synovial Mucin By KARL MEYER (by invitation) and M. H. DAWSON New York, N. Y.

Although the importance of synovial mucin in the physiology of joints is generally recognized its chemical nature has not been established. By a method similar to that employed by one of the authors (K. M.) in the isolation of chondroitin-sulfuric acid a polysaccharide acid of high molecular weight has been obtained which possesses most of the viscosity of the starting material. This polysaccharide acid consists of equimolar parts of hexosamine, hexuronic acid and acetyl the latter apparently as N-acetylglucosamine. The polysaccharide occurs in synovial fluid either free or united to protein in salt linkage only.

In composition and rotation the polysaccharide acid appears to be identical with the polysaccharide acid of vitreous humor, umbilical cord and the mucoid phase of Group A hemolytic streptococci. Further evidence of the identity of these polysaccharides is provided by the fact that they are hydrolyzed at the same rate by a specific enzyme.

The protein component of the carbohydrate-protein complex appears to be a globulin which forms an insoluble salt with the acid polysaccharide on acidification.

Immunological aspects of the polysaccharide acids obtained from various sources will be briefly considered.

Plasma Specific Gravity as an Aid in the Estimation and Maintenance of a Safe Fluid Balance in Artificial Fever By HERBERT R. BROWN JR., WILLIAM F. CLARK, and NATHANIEL JONES (by invitation), and STAFFORD L. WARREN Rochester, New York.

Adequate storage and replacement of reserve water and electrolytes is necessary to safeguard the patient against dehydration resulting from increased losses of these substances during artificially induced fever.

Fluctuations in plasma specific gravity have been observed in 50 cases under various conditions (preparation for fever treatment, compensation during treatment and sequelae of treatment).

There seems to be a good correlation between plasma specific gravity values and the clinical findings of adequate hydration, overhydration and dehydration and collapse states.

During adequate hydration plasma specific gravity fluctuates within the normal range defined by Van Slyke. Clinically the patient is in good condition (normal blood pressure, frequent urine excretion, moderately profuse sweating, absence of mania somnolentia).

In states of dehydration accompanied by shock, blood pressure falls, anuria, lack of sweating, mania and collapse, the plasma specific gravity rises well above normal levels.

In overhydration plasma specific gravity falls below normal levels in keeping with the clinical evidence (blood pressure rises, polyuria, edema, excessive sweating, restlessness, etc.).

Plasma specific gravity values change significantly before the clinical symptoms of dehydration or overhydration are clearly evident so that the hydration of the

patient may be safely and quickly adjusted in the necessary direction.

Protective Antibodies in the Serum of Human Syphilis By THOMAS B. TURNER and (by invitation) WILLIAM L. FLEMING and NANCY L. BRATTON Baltimore, Md.

In a previous paper experiments were reported which showed that during the course of experimental syphilis rabbits developed protective antibodies against virulent *Treponema pallidum* and that the presence of these antibodies was associated with a high degree of resistance to reinfection (Turner T. B., J. Exper. Med.—in press). The present paper reports the results of protection tests made on the serum of 80 persons, 60 of whom had or had had syphilis, and 20 of whom were presumably non-syphilitic.

The technique of the test was the same as that used for testing rabbit sera. One part of a tissue emulsion rich in virulent *T. pallidum* was combined with 9 parts of whole serum, the mixture incubated for 6 hours at 37° C. and inoculated intracutaneously in 6 sites of one area in each of 4 normal rabbits. Sera were tested in groups of 4, one serum of each group being from a presumably non-syphilitic person. The same lot of spirochete emulsion which had been preserved by freezing was employed in all hot one group of tests. Protection was manifested by failure of syphilitic lesions to develop at the sites of inoculation or by a prolonged incubation period as compared with the lesions in the control areas.

Of 60 sera from persons with syphilis, 53 showed definite protection, in 4 the results were equivocal and in 3 there was no evidence of protective antibodies. Of 20 sera from presumably non-syphilitic persons, 16 showed no evidence of protection, in 2 the results were equivocal and 2 showed definite protection. Of 11 syphilitics with negative Wassermann tests, the sera of 10 contained protective antibodies.

The bearing of these findings on the question of humoral immunity in syphilis and the relationship of protective antibodies to the ordinary diagnostic serological tests were briefly discussed.

The Bactericidal Effect of Sulfanilamide upon Pathogenic and Non Pathogenic Staphylococci By WESLEY W. SPINK, Minneapolis, Minn.

The purpose of this study was to show that sulfanilamide has a definite bactericidal effect upon staphylococci and also to demonstrate certain factors influencing the effect of sulfanilamide upon bacteria. Twelve pathogenic and seven non pathogenic strains were included. The influence of each of the following factors on the bactericidal action of sulfanilamide was observed: the nature of the culture media with special reference to ingredients and pH; the strain of staphylococcus; the number of organisms in suspension; the concentration of sulfanilamide; the temperature at which suspensions of organisms and the drug were incubated; the length of time necessary for sulfanilamide to exert its effect.

Employing the usual type of yeast infusion broth as media, high concentrations of

100 organisms When sterile, human urine was substituted as a culture media, a marked bactericidal effect was obtained with low concentrations The bactericidal effect was much more pronounced at an incubation temperature of 40° C than at 37° C When only one drop of veal infusion broth was added to 5 cc of urine, the foregoing action of sulfanilamide was greatly inhibited These observations indicate that peptone or peptone-like substances may inhibit the action of sulfanilamide, and that an elevation of temperature within human physiological limits is desirable for an optimum effect

The Use of Sulfapyridine in the Treatment of Pneumonia

By J MURRAY KINSMAN and JOHN WALKER MOORE,
Louisville, Ky

During the winter and early spring of 1939, we treated, in the Louisville City Hospital, 40 cases of pneumonia with sulfapyridine with a mortality rate of 5 per cent The pneumonias have been of both the lobar and bronchial variety Many different types of pneumococcus have been recovered from the sputum

In every case, blood concentration curves have been obtained for both free and total sulfapyridine Blood was taken at hourly intervals for 4 hours after the initial dose, and thereafter, once daily throughout the course of treatment and until the drug disappeared from the blood stream Daily urines have also been examined quantitatively for the excretion of the drug in both the free and conjugated form

The results of the blood and urine studies in general agree with the results of other workers It appears that a blood concentration of the free sulfapyridine of anywhere from 1 to 12 may be effective in curing pneumonia Absorption is very irregular A few cases had recurrences of fever after the drug was discontinued and return of the temperature to normal when it was started again No toxic effects on the blood were noted Two patients had severe hematuria with complete recovery when the drug was stopped Nausea was a very common side-effect Empyema was not cured, even though the concentration of the sulfapyridine in the empyema fluid was much higher than in the blood

Hemolysis from Sulfapyridine By LOWELL A ERF and COLIN M MACLEOD (introduced by C P Rhoads),
New York, N Y

The widespread and effective use of sulfapyridine in pneumonia has made important a knowledge of its possible harmful effects Furthermore, observations of the hematological changes caused by chemicals of known constitution are useful for understanding the hematopoietic disorders of unknown etiology

The urinary and fecal excretions of urobilin have been measured quantitatively, as an index of the rate of hemolysis, in 20 cases of pneumonia treated with sulfapyridine As controls, 6 febrile cases of pneumonia, treated by the drug, have been studied similarly The usual observations of the blood cells were made in all instances

The results of the study are striking The patients

treated with large amounts of sulfapyridine showed unequivocal evidence of an increased rate of hemolysis From two to ten times the normal amounts of urobilin were excreted, the greatest amounts by the patients receiving the most drug Concurrently there appeared anemia, leukocytosis, and reticulocytosis, furthermore, in certain instances a diversion of urobilin from feces to urine gave evidence of hepatic insufficiency When treatment with sulfapyridine was discontinued all evidence of abnormal blood destruction disappeared Patients receiving moderate amounts of the drug excreted slightly increased amounts of urobilin, but when little drug was given no sign of abnormal hemolysis could be detected Control cases without medication with sulfapyridine showed no increased excretion of urobilin

It is concluded that sulfapyridine in large amounts causes an increase of the hemolytic process, although the disease for which the drug is given does not do so The effect seems to be roughly proportional to the amount of drug administered

Activity in the Central Nervous System During Anesthesia

By HENRY K BEECHER (introduced by E D Churchill),
Boston, Mass

Investigation of the anesthesia process has been handicapped by lack of precisely measurable elements The electrical activity of the central nervous system, and in particular that of the cerebral cortex, presents a suitable component for study Measurement of the electrical activity there has been made possible by recent advances in electrophysiology The application of the technics of this science to study of the central nervous system during anesthesia not only gives information leading to a more complete understanding of cortical action potentials but also presents fundamental information as to the characteristics of the anesthesia process itself

When the frequency and voltage of the electrical waves and the pattern of the activity are considered in conjunction with the response to peripheral stimulation under the several agents at various levels of anesthesia, it becomes possible to make certain statements The frequency of the cortical waves is characteristic for a given agent It is a constant over a wide range of anesthesia depth On the other hand, voltage of the waves is labile Voltage is directly related to anesthesia depth In one group of agents, but not in another, voltage can be altered by peripheral (sciatic) stimulation Under deep anesthesia sciatic stimulation has no effect on voltage under any agent The seventeen anesthetic agents studied can be sharply divided into two groups on the basis of six criteria: volatility, frequency of cortical waves, pattern, presence or absence of a secondary cortical discharge following peripheral stimulation, response of the voltage of cortical waves to sciatic stimulation, and type of flexion reflex A consideration of these points leads to a mass of objective data regarding the central nervous system effects of various anesthetic agents and permits a general hypothesis to be stated as to the fundamental difference of action of volatile and non-volatile agents

Basis of the Hematopoietic Activity in Pernicious Anemia of Decalcified Hog Ileum By SMITH O. DEXTER, ROBERT W. HEINLE and HERBERT J. FOX (by invitation) and WILLIAM B. CASTLE, Boston, Mass.

Increased blood production in pernicious anemia has been observed by various workers to follow the oral administration not only of decalcified hog stomach but also of similar preparations of the duodenum, jejunum, ileum and even the colon of that animal. From this evidence others have concluded that the so-called intrinsic factor is secreted by the intestine as well as by the stomach or that the intestine plays an active part in the subsequent preparation or storage of an autohemopoietic principle.

Still another interpretation is however possible, because these portions of the alimentary tract are normally exposed to gastric secretions from above. Their blood-forming activity might therefore be due entirely to passive adsorption of intrinsic factor of gastric origin. If this were so it might be more readily removed by prolonged washing than if actually secreted by the mucosa. Accordingly a stream of cold running water was caused to distend and to pass through in different experiments several fresh specimens of the stomach, the duodenum and jejunum (3 feet) or the lower half (15 feet) of the small intestine of the hog. After 6 hours of such washing the entire mucosa was separated from the muscular layer and ground with approximately 400 grams of finely divided beef muscle per 100 feet of intestine. In another series of preparations of duodenum and stomach the mucosa was finely minced and suspended in a large volume of cold water which was continuously agitated by an inflowing stream. After 6 hours the mucosa was filtered off, excess water removed and ground with finely divided beef muscle. The approximate proportions, 400 grams of beef muscle to the mucosa of each of six stomachs and 400 grams per 100 feet of duodenum were used. The mixtures were then dried in a current of warm room air defatted and pulverized. As controls similar preparations of the various organs were made, except for the prolonged washing.

Observations on 12 patients with typical Addisonian pernicious anemia were made. The hematopoietic activity of unwashed preparations of ileum, previously observed by others, was entirely confirmed. Its blood-forming power like that of the hog stomach was, however, found to be readily destroyed by boiling and thus shown not to be due to active material similar to that in liver. A washed preparation of hog's stomach retained its hematopoietic activity but the lower half of the small intestine after washing possessed in three instances no detectable, and in one instance only slight blood-forming activity. Washing did not remove the activity of the duodenal mucosa but mincing and then washing rendered inactive the duodenal mucosa but not that of the stomach.

It is therefore suggested that the hematopoietic power of the "unwashed" ileum of the hog is due to the passive adsorption of gastric secretion. Whether the activity of the washed duodenum of the hog is due to an active

secretion of intrinsic factor as has been assumed by others, or to the inability of the washing process to get rid of a higher local concentration of adsorbed intrinsic factor remains uncertain. Variations in the texture and thickness of the mucosa of the various organs cannot be excluded as a cause of the differences in hematopoietic activity especially following the mincing and washing procedure.

A Newly Recognized Granulopenic Syndrome Caused by Excessive Splenic Leukolysis and Successfully Treated by Splenectomy By B. K. WISEMAN (by invitation) and C. A. DOAN, Columbus, Ohio

Theoretically from the accumulated knowledge of splenic physiology there might be expected to occur a primary more or less specific, granulopenic syndrome, comparable to congenital hemolytic jaundice or thrombocytopenic purpura, to which the spleen pathologically segregates and destroys leukocytes instead of red cells or platelets.

Practically such a syndrome has been encountered during the past year in an acute, as well as in a sub-acute and chronic, form. The mechanism was established by direct sternal marrow studies revealing in each instance myeloid hyperplasia of qualitatively normal cells by gross splenomegaly with profound peripheral granulopenia by ruling out associated liver cirrhosis (Banti's syndrome), chronic infection and other contributing organic, drug or environmental factors and finally by the therapeutic test of splenectomy which was followed by a prompt re-establishment of a normal peripheral white cell count.

Histologically each of the three spleens removed showed extreme clasmotocytosis with excessive phagocytosis of granulocytes.

Chemical extracts of the splenic tissue have been injected into rabbits and monkeys in an attempt to reproduce the syndrome experimentally.

The Experimental Production of Congestive Splenomegaly: Preliminary Report By LOUIS M. ROUSSELOT (by invitation) and WILLIAM P. THOMPSON, New York, N. Y.

In an attempt to produce a congestive splenomegaly (Banti's syndrome) in dogs, various chronic hepatic irritants have been tried.

If a sterile suspension of 1 to 1.5 grams of SiO_2 particles, measuring 1 to 3 micra in size, is introduced into the dog's splenic vein the particles promptly clear through the liver and appear in the hepatic lymph nodes. However, if after several months silica is again injected the particles remain in the liver where they produce a slowly progressive, periportal nodular fibrosis. A total of 60 grams, injected in divided doses, will result in a widespread cirrhosis, by the end of the second year. With the development of this hepatic lesion portal hypertension and congestive splenomegaly appear, the splenic vein pressures rising to over 250 mm. of H_2O , the spleens increasing to from 4 to 6 times their normal size. Anemia and thrombocytopenia appear and in the one animal autopsied an esophageal varix was present.

Fatal Probable Riboflavin Deficiency in Man By HENRY FIELD, JR., and (by invitation) EDWIN C. WISE, Ann Arbor, Mich

Until recently, nothing has been known concerning the function of riboflavin in man. Sebrell and his coworkers have studied riboflavin deficiency in dogs. They reported sudden collapse and coma, promptly followed by death unless adequate doses of riboflavin were given. The outstanding necropsy finding was a yellow mottling of the liver and fatty infiltration of the liver and of the tubules of the kidneys.

We have observed patients who died unexpectedly and who, at autopsy, had similar fatty livers and kidneys. The dried liver of one patient has been found by biological assay to contain 10 Sherman units of vitamin B₂ per gram. The Vourquin-Sherman assay method is believed generally to measure riboflavin. The value found is of the order of magnitude found in experimental animals dying of riboflavin deficiency.

Fatty infiltration of the liver is a very common finding in patients dying of chronic disease. It was found to be much more marked in a series of autopsies of patients dying of ulcerative colitis than in a series of patients dying within 24 hours after accidents. It is suggested that a deficiency of the vitamins which are stored in relatively small amounts is an important factor in the death of such patients.

In Vitro and In Vivo Studies on the Dissolution of Phosphatic Urinary Calculi with Citrate Solutions By FULLER ALBRIGHT and (by invitation) HIRSH W. SULKOWITZ, Boston, Mass

This study was undertaken with the ultimate purpose of finding a solution which would dissolve phosphatic renal calculi on direct introduction of the solution into the renal pelvis. It was found that acid citrate solutions would dissolve phosphatic urinary calculi in a surprisingly short period of time in *in vitro* experiments. In order to study the quantitative effects of pH, of concentration of citrate, and of temperature it was found necessary to have a substance of uniform composition. Crystals of the mineral francolite were used for these solubility studies.

A case in which large bladder calculi were dissolved by a citrate solution introduced and withdrawn from the bladder by means of a constant tidal drainage apparatus will be reported.

Radioactive Iodine as an Indicator of Thyroid Physiology: Observations on Animal and Human Subjects By S. HERTZ, A. ROBERTS, and R. D. EVANS (by invitation), and J. H. MEANS, Boston, Mass

By means of artificially produced radioactive iodine, the authors were able to label various dosages of this element and study their fate in both animals and man. The distribution in tissues, excreta, and body fluids was determined. The quantity of iodine concentrated within the thyroid in various functional states, including Graves' disease, was investigated. The partition of the thyroid iodine among several chemical fractions was studied with

the cooperation of Dr. William T. Salter, of the Huntington Memorial Hospital in Boston.

The rate of absorption and excretion of radioactive iodine can be taken as an index of that of ordinary iodine, since the radioactive isotopes differ in no chemical manner from stable isotopes. This method of study offers advantages of accuracy exceeding those of any chemical methods of iodine analysis so far applied to any clinical or biological problems.

Respiratory Metabolism of Human Muscle By EDGAR S. GORDON, MARC J. MUSSER, and IRENE STARK (introduced by E. L. Sevringhaus), Madison, Wis

Because older methods of investigation of the myopathies have failed to reveal the true nature of any of these diseases, a totally different approach has been made to this problem through a study of the respiratory metabolism of human muscle, as measured with the Warburg and Barcroft types of respirometer. Small sections of muscle for study were obtained at biopsy from the gastrocnemius. Normal muscle, used as control material, was obtained in the operating room from individuals undergoing surgery for a variety of conditions, in none of which was there any reason to suspect a disturbance of metabolism. This technique has made possible a study of the enzyme systems involved in the energy metabolism of muscle.

It seemed most logical first to repeat with human muscle some of the well established animal work. The important finding of Krebs on the *in vitro* action of insulin as a catalyst of intracellular respiration has been confirmed in diabetic human muscle. A failure to oxidize succinate has been found to occur in muscle from two patients with myasthenia gravis, but the exact mechanism of this defect has not been determined, and the phenomenon has not been observed in all cases of this disease. In one case of myotonia atrophica and in one case of amyotrophic lateral sclerosis, the addition of pyruvate as a substrate caused a marked increase in respiration, which does not occur in controls.

The effects of various anesthetics were established and found to be unimportant except with the use of pyruvate as substrate with cyclopropane anesthesia. These studies have provided suggestive evidence of chemical abnormalities in pathological muscle and have given some helpful indications for the planning of further investigations which are now in progress.

Migraine of the Vasodilating Type: Treatment with Histamine By BAYARD T. HORTON and (by invitation) ALEXANDER H. MACLEAN and WINCHELL MCK. CRAIG, Rochester, Minn

The results of treatment of 84 patients with migraine of the vasodilating type by "desensitization" with histamine are presented. In this study, we have considered migraine as a functional vascular disease. We recognized two types, the vasoconstricting and the vasodilating. The vasoconstricting type is usually familial, begins in early life and is characterized by severe headache, frequently of the hemicranial type, which usually is associated with nausea, vomiting, and ophthalmic symptoms. The

vasodilating type begins later in life, is usually of the hemispherical type and is associated with evidence of vasodilatation watering of the eye, and blockage of the nostril on the affected side. Nausea vomiting and scotomas are invariably absent.

During the past 18 months, we have observed 84 patients with migraine of the vasodilating type. Only patients who were refractory to other forms of treatment and who were having from 2 to 20 attacks a week were selected for study. They were under observation for 2½ to 4 weeks. Detailed observations and experimental studies were carried out. We were able to produce attacks of migraine of the vasodilating erythromelalgic type experimentally by the administration of histamine and other vasodilating agents and to control the attacks completely by the administration of adrenalin and other vasoconstricting agents. In 2 cases, immersion of the hand of the patient in cold water at 4 C for 1 minute caused a resultant rise in blood pressure which in turn brought about an abrupt cessation of the attack of pain. Patients were unable to distinguish between induced and previous spontaneous attacks. During the induced as well as during the spontaneous attacks, a rise of 1 to 3 C in surface temperature of the involved region was observed. These observations were made with electric thermocouples while the patient was under controlled environmental conditions.

The method of treatment consists in giving subcutaneously 0.05 mgm. of histamine twice a day for 2 consecutive days. On the third day the dose is increased to 0.066 mgm. twice a day and by the fifth day 0.1 mgm. twice a day is well tolerated. The injection of 0.1 mgm. twice a day is continued for 2 to 3 weeks.

Sixty five patients obtained definite permanent relief for periods of 2 weeks to 18 months. Several of these patients have suffered from a recurrence of their symptoms but these recurrences promptly responded to another course of treatment with histamine. In cases in which treatment has been employed recently we have been giving 0.1 mgm. of histamine subcutaneously at weekly intervals whenever possible in an attempt to prolong the period of freedom from attack. Ten patients received no benefit from administration of histamine for 2 weeks and 9 patients have not been heard from since their dismissal from our care.

In order to determine the rationale for the effect of histamine the size of the wheal and flare following the intracutaneous injection of histamine of varying dilutions has been used as an index of the patient's response to the treatment by Brown and one of us (Hortoo). In 45 per cent of the cases studied the flare that appeared in 100 per cent of the cases before treatment with histamine was begun could not be reproduced after treatment. Since formation of the flare is dependent on the axon reflex, the daily injections of histamine in some way have altered this reflex mechanism.

Guinea pigs have been given relatively large doses of histamine subcutaneously twice a day for 2 to 3 weeks by one of us (Horton) and Essex, and later these animals have been given intravenously approximately twice the calculated lethal dose of histamine for guinea pigs. Approx-

mately 50 per cent of the guinea pigs which had received previous injection of histamine recovered whereas only 13 per cent of the control guinea pigs recovered. Hypertrophy of the adrenal glands occurred in the guinea pigs previously treated with histamine.

Adrenocortical Function in Hypopituitarism By D J STEPHENS Rochester N Y

Anatomical findings in hypophysectomized animals and in patients with hypopituitarism would indicate that impairment of adrenocortical function occurs in hypophyseal insufficiency and if so one might expect to find disturbances of electrolyte balance in pituitary disease. With this in mind a study of the chloride excretion of a group of patients with hypopituitarism was made. The modification of the chloride depletion test recently described by Cnifer Powers, and Wilder was used. Six of seven patients with clinical evidence of well established hypopituitarism showed increased concentration of salt to the urine similar to that which has been found to be characteristic of adrenocortical insufficiency. Four of the six patients developed symptoms suggesting those of an Addisonian crisis. These symptoms were relieved by the intravenous administration of sodium chloride and adrenal cortex extract. In two patients symptoms failed to occur and the results of the test were favorably modified after the administration of extra sodium chloride and adrenal cortex extract.

The Assay of Desoxycorticosterone Acetate and its Use in the Treatment of Addison's Disease By R. A. CLEGGORN and (by invitation) J. L. A. FOWLER and J. S. WENZEL, Toronto Can.

The minimal amount of desoxycorticosterone acetate in oil (Ciba) necessary to maintain the blood nonprotein nitrogen within normal limits has been determined on four adrenalectomized dogs. The standard diet used consisted chiefly of lean meat and contained approximately 2 per cent sodium chloride by dry weight. The hormone requirement varied from 0.031 to 0.17 mgm. per kgm. per dog per day.

Seven patients with Addison's disease have been successfully treated with desoxycorticosterone acetate. Four had previously been receiving sodium salts and so aqueous extract of adrenal cortex. It was found in four cases that 1 cc. of the desoxycorticosterone (5 mgm.) given intramuscularly on alternate days was a more effective maintenance dose than 5 cc. of the extract previously used. Blood chemical values were maintained within normal limits and the patients voluntarily stated that they experienced a greater sense of well being and vigor while receiving desoxycorticosterone acetate than previously. Signs of excessive dosage were observed in one case.

The Treatment of Adrenal Insufficiency with Desoxycorticosterone Acetate (A Synthetic Adrenal Cortical Hormone) By GEORGE W. THORN and (by invitation) R. PALMER HOWARD and KENDALL EMERSON JR. Baltimore, Md.

Ten patients with Addison's disease received daily injections of 5 to 30 mgm. of desoxy-corticosterone acetate.

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During the past 18 months we have observed 84 patients with migraine of the vasodilating type. Only patients who were refractory to other forms of treatment and who were having from 2 to 20 attacks a week were selected for study. They were under observation for 2½ to 4 weeks. Detailed observations and experimental studies were carried out. We were able to produce attacks of migraine of the vasodilating, erythromelalgic type experimentally by the administration of histamine and other vasodilating agents and to control the attacks completely by the administration of adrenalin and other vasoconstricting agents. In 2 cases immersion of the hand of the patient in cold water at 4° C. for 1 minute caused a resultant rise in blood pressure which in turn brought about an abrupt cessation of the attack of pain. Patients were unable to distinguish between induced and previous spontaneous attacks. During the induced as well as during the spontaneous attacks a rise of 1 to 3° C. in surface temperature of the involved region was observed. These observations were made with electric thermocouples while the patient was under controlled environmental conditions.

The method of treatment consists in giving subcutaneously 0.05 mgm. of histamine twice a day for 2 consecutive days. On the third day the dose is increased to 0.066 mgm. twice a day and by the fifth day 0.1 mgm. twice a day is well tolerated. The injection of 0.1 mgm. twice a day is continued for 2 to 3 weeks.

Sixty-five patients obtained definite permanent relief for periods of 2 weeks to 18 months. Several of these patients have suffered from a recurrence of their symptoms, but these recurrences promptly responded to another course of treatment with histamine. In cases in which treatment has been employed recently we have been giving 0.1 mgm. of histamine subcutaneously at weekly intervals whenever possible in an attempt to prolong the period of freedom from attack. Ten patients received no benefit from administration of histamine for 2 weeks and 9 patients have not been heard from since their dismissal from our care.

In order to determine the rationale for the effect of histamine the size of the wheal and flare following the intracutaneous injection of histamine of varying dilutions has been used as an index of the patient's response to the treatment by Brown and one of us (Hortoo). In 45 per cent of the cases studied the flare that appeared in 100 per cent of the cases before treatment with histamine was begun could not be reproduced after treatment. Since formation of the flare is dependent on the axon reflex, the daily injections of histamine in some way have altered this reflex mechanism.

Guinea pigs have been given relatively large doses of histamine subcutaneously twice a day for 2 to 3 weeks by one of us (Hortoo) and Essex, and later these animals have been given intravenously approximately twice the calculated lethal dose of histamine for guinea pigs. Approx-

mately 50 per cent of the guinea pigs which had received previous injection of histamine recovered whereas only 13 per cent of the control guinea pigs recovered. Hypertrophy of the adrenal glands occurred in the guinea pigs previously treated with histamine.

Adrenocortical Function in Hypopituitarism By D. J. STEPHENS, Rochester, N. Y.

Anatomical findings in hypophysectomized animals and in patients with hypopituitarism would indicate that impairment of adrenocortical function occurs in hypophyseal insufficiency and if so one might expect to find disturbances of electrolyte balance in pituitary disease. With this in mind a study of the chloride excretion of a group of patients with hypopituitarism was made. The modification of the chloride depletion test recently described by Cotler, Powers and Wilder was used. Six of seven patients with clinical evidence of well established hypopituitarism showed increased concentration of salt in the urine similar to that which has been found to be characteristic of adrenocortical insufficiency. Four of the six patients developed symptoms suggesting those of an Addisonian crisis. These symptoms were relieved by the intravenous administration of sodium chloride and adrenal cortex extract. In two patients symptoms failed to occur and the results of the test were favorably modified after the administration of extra sodium chloride and adrenal cortex extract.

The Assay of Desoxycorticosterone Acetate and its Use in the Treatment of Addison's Disease By R. A. CLEGGON and (by invitation) J. L. A. FOWLER, and J. S. WENZEL, Toronto, Can.

The minimal amount of desoxycorticosterone acetate in oil (Ciba) necessary to maintain the blood nonprotein nitrogen within normal limits has been determined in four adrenalectomized dogs. The standard diet used consisted chiefly of lean meat and contained approximately 2 per cent sodium chloride by dry weight. The hormone requirement varied from 0.031 to 0.17 mgm. per kgm. per dog per day.

Seven patients with Addison's disease have been successfully treated with desoxycorticosterone acetate. Four had previously been receiving sodium salts and an aqueous extract of adrenal cortex. It was found in four cases that 1 cc. of the desoxycorticosterone (5 mgm.) given intramuscularly on alternate days was a more effective maintenance dose than 5 cc. of the extract previously used. Blood chemical values were maintained within normal limits and the patients voluntarily stated that they experienced a greater sense of well being and vigor while receiving desoxycorticosterone acetate than previously. Signs of excessive dosage were observed in one case.

The Treatment of Adrenal Insufficiency with Desoxycorticosterone Acetate (A Synthetic Adrenal Cortical Hormone) By GEORGE W. THORN and (by invitation) R. PALMER HOWARD and KENDALL EMERSON, JR., Baltimore, Md.

Ten patients with Addison's disease received daily injections of 5 to 30 mgm. of desoxy-corticosterone acetate.

Despite sodium chloride restriction this treatment resulted in marked clinical improvement and was associated with an increase in plasma volume, body weight and blood pressure, sodium and chloride retention, and an increased renal excretion of potassium. Withdrawal of treatment (48 to 96 hours) was followed by the onset of signs and symptoms of adrenal insufficiency and resumption of treatment resulted in rapid recovery.

Under local anesthesia, pellets (75 to 150 mgm) composed only of crystals of desoxy-corticosterone acetate were implanted subcutaneously, in the infrascapular regions in six patients with Addison's disease. Treatment by this method resulted in marked clinical improvement, an increase in body weight and blood pressure, the maintenance of a normal concentration of serum electrolytes, and a positive sodium and chloride balance. The number of pellets necessary for maintenance was estimated from the daily requirement of desoxy-corticosterone acetate in oil. At intervals pellets were removed and weighed and the quantity of hormone absorbed was determined. The implantation of pellets obviated the necessity for daily injections and resulted in a considerable saving in hormone (30 to 40 per cent). A single implantation of pellets was found to provide adequate hormone therapy for several months.

Conclusions The effect of desoxy-corticosterone acetate treatment is similar to that of adrenal cortical extract. The implantation of pellets of crystalline desoxy-corticosterone acetate appears to be feasible, and an economical and effective treatment for patients with Addison's disease.

The Effects on the Cardiovascular System in Man of Benzedrine (Amphetamine) and Paredrine By M. D. ALTSCHULE and A. IGLAUER (introduced by S. L. Gargill), Boston, Mass.

The effects of amphetamine sulfate (benzedrine sulphate) and paredrine bromide given in doses of 10 to 70 mgm on the cardiovascular system were studied in 15 normal human subjects. The drugs were given by mouth or by intramuscular injection. In three cases the effects of adrenalin were also studied.

Amphetamine (benzedrine) and paredrine caused a marked rise in systolic and diastolic blood pressures, the latter being somewhat more effective than the former. The cardiac output, pulse rate, pulmonary circulation time, venous pressure, and vital capacity were not changed. In several instances transitory slowing of the pulse occurred at the onset of the rise in arterial blood pressure due apparently to a vagal reflex. In such cases there was also a transitory slight fall in cardiac output.

The effects of adrenalin were quite different, consisting in a slight rise in systolic blood pressure, no change or a fall in diastolic blood pressure, marked increase in pulse rate and cardiac output, and marked shortening of the pulmonary circulation time.

These findings suggest that paredrine may be a useful drug in the treatment of certain types of shock in which stimulation of the myocardium may be undesirable. These include the shock of diabetic coma, overwhelming infection,

and cardiac infarction. Paredrine is the drug of choice inasmuch as it has none of the stimulating effect on the cerebral cortex associated with the administration of amphetamine (benzedrine).

The Precordial Electrocardiogram in Myocardial Infarction Complicated by Bundle Branch Block By FRANKLIN D. JOHNSTON, FRANK N. WILSON and (by invitation) HANS HECHT, Ann Arbor, Mich.

It is well known that bundle branch block either in transient or permanent form often occurs after a coronary occlusion and that it may modify the ventricular deflections of the standard leads so profoundly as to completely mask the characteristic QRS and T-wave changes of infarction. This situation is especially apt to arise when left bundle branch block is present and under these circumstances precordial leads also fail to show the usual QRS changes of anterior myocardial infarction unless, as occurred in one of our cases, the infarct extends entirely through the lower end of the interventricular septum.

When right bundle branch block is present, recognizable QRS changes of anterior infarction are often seen in standard Lead I, and abnormally large Q deflections are found in serial precordial leads.

Quantitative Measurement of Cardiac Stroke and Valvular Leakage in Man By ANCEL KEYS and H. L. FRIEDEL (introduced by C. J. Watson), Minneapolis, Minn.

Both systolic and diastolic areas of the heart can be measured on plates made with the multiple-slit roentgenkymograph. A method was devised in which roentgenkymographic plates were made simultaneously with acetylene-rebreathing measurements of cardiac output. From 21 experiments on 13 normal subjects we found stroke

$$\text{volume} = 0.64 \left(\frac{\text{area}_{\text{diastole}}}{1.45} - \frac{\text{area}_{\text{systole}}}{1.45} \right)$$

The coefficient of correlation between the two estimates of stroke volume was $+0.984$. The mean discrepancy was ± 5.1 per cent, the maximum errors were $+10.7$ and -12.2 per cent. Similar results were obtained in myxedema and simple hypertension.

Nine patients with aortic or mitral regurgitation were studied. The gross output of the heart exceeded the net stroke output in all cases. The indicated valvular leaks ranged from 10 to 50 per cent of the gross stroke, and these values were closely parallel to the clinical findings. Repetitions showed that these estimates of leakage are relatively constant. All evidence indicates that our procedure accurately measures (1) total heart volume, (2) total heart stroke, (3) net circulation, and (4) the volume of blood regurgitated.

The Effect of Congestive Heart Failure on Erythropoiesis and Blood Pigment Metabolism By JOHN V. WALLER (by invitation) and HERRMAN L. BLUMGART, Boston, Mass.

Studies of the effect of congestive failure and its attendant anoxia on the red cells and pigment metabolism and the relation of erythropoiesis and hemolysis to changes in the blood volume have been made in thirteen subjects. Before congestive failure becomes pronounced, there is a

sympathectomy as ordinarily practiced for Raynaud's disease of the upper extremities consists of ganglionectomy, that is, postganglionic section. They have maintained that failure to cure Raynaud's disease in the upper extremities results because postganglionic sympathectomy increases sensitivity to circulating epinephrine much more than preganglionic sympathectomy performed for the lower extremities does. Our studies indicate that lumbar ganglionectomy (preganglionic section) does not increase sensitivity of the arterioles of the toes to epinephrine significantly. Both preganglionic section and postganglionic section of the sympathetic nerves to the hands increase the sensitivity of the digital arterioles to intravenous injection of epinephrine and in about the same degree. Therefore what appeared to be a pleasant physiological explanation for the peculiar fact that Raynaud's disease of the feet may be cured routinely by sympathectomy while Raynaud's disease of the hands is not cured routinely by sympathectomy has been reexamined and found to be of very uncertain value.

The Preparation of Membranes of Graded Permeability from Cellophane and Their Use as a Means of Measuring Relative Molecular Size By W. B. SEYMOUR (introduced by J. M. Hayman, Jr.) Cleveland, Ohio

An elaboration of the method of McBain and Stuewer for increasing the permeability of DuPont Cellophane by immersion in zinc chloride solutions has been developed. This permits the preparation of membranes of graded permeability. The increase in permeability is proportional within certain limits to the concentration and the temperature of the zinc chloride solutions. The change in the membrane is probably in the nature of a physical rearrangement of the cellulose structure, not or very little imbibition of water taking place.

The advantages of cellophane over the collodion membranes used by others for measuring molecular size are that the former are prepared quickly and easily and they do not absorb protein.

An estimation of the pore size is obtained by means of Poiseuille's law. Membranes so calibrated have been used in ultrafiltering normal human plasma, acacia oxyhemoglobin, and crystalline egg albumen. The results indicate that the size of the pores is relatively uniform and that the molecules of oxyhemoglobin and crystalline egg albumen are nearly spherical and homodisperse. Osmotic solutions show polydispersion of molecules. Using plasma membranes of proper size will allow the passage of about 40 per cent of the albumin fraction, holding back the remaining albumin and all of the globulin. The order of relative size of the above is plasma albumin < egg albumen < acacia < plasma globulin < oxyhemoglobin.

Spherocytosis (and Increased Erythrocyte Fragility) as Indicators of Hemolytic Activity with a Consideration of Differential Fragility By WILLIAM DAMESHEK and (by invitation) STEVEN O. SCHWARTZ and KARL SINGER, Boston, Mass.

Our previous studies demonstrated that spherocytosis (and increased saline fragility) is not pathognomonic of

congenital hemolytic jaundice but is common to various hemolytic syndromes. Present studies demonstrate that this phenomenon may be produced experimentally by various types of hemolytic agents either *in vitro* or *in vivo*. Distilled water, saponin, phenylhydrazine, lysolecithin, or immune hemolytic serum produced spherocytosis of varying degree, dependent primarily upon dosage. Studies of the bone marrow and of diameters of both reticulocytes and mature red cells indicate that the spherocyte is a mature erythrocyte which has been morphologically altered outside of the bone marrow. Spherocytosis may thus be utilized as a morphological indicator of increased hemolytic activity. The degree of spherocytosis is in general proportional to the rapidity of the hemolysis.

Although all spherocytes, however produced, present increased fragility to hypotonic solutions of sodium chloride (a physicochemical phenomenon) their behavior to other lysins such as saponin and lysolecithin differs in different experimental and pathological conditions. In other words, although spherocytes are morphologically similar they may differ physiologically depending upon the type of lysis which has already acted upon the red cell. Preliminary observations in various disease states suggest that the reaction of the spherocyte to various substances (differential fragility) may be helpful in the differentiation of the various types of hemolytic anemia.

Studies of the Circulation in a Group of College Athletes By HAROLD J. STEWART and (by invitation) ROBERT F. WATSON, New York, N. Y.

In this study the following measurements were carried out on a group of 14 college athletes who at the time were members of a University football team: arteriovenous-oxygen difference, oxygen consumption, minute volume, output, cardiac work, vital capacity, cardiac size, circulation time, venous pressure, arterial pressure, and heart rate. Similar observations were made of a control group of healthy young men of the same age group engaged in ordinary activities. The average values obtained for the athletes were as follows: arteriovenous-oxygen difference equals 63.9 cc. per liter; cardiac output (index) measured 2.12 liters per square meter of body surface area; per minute stroke volume equals 65 cc.; stroke volume per kilogram of body weight equals 0.80 cc.; left ventricular work per beat equals 1.06 grammeters per kilogram of body weight; venous pressure equals 9.2 cm. of saline circulation time (dechlorin arm to tongue) equals 15.4 seconds; and the cardiothoracic ratio equals 42.9 per cent. These values were compared with those for the control group and the only significant difference appeared in the stroke volume. This difference, however, is believed to be the result of a difference in the average size (weight) of the two groups. Electrocardiograms showed no variation from the normal in either group. It is concluded that the circulation in this group of college athletes as measured by these methods, shows no appreciable variation from that in a group of normal young adult males.

In 3 patients with obstructive jaundice, blood iodine of 470 micrograms per cent, 1167 micrograms per cent, and 488 micrograms per cent were obtained 20 days, 20 days, and 30 days respectively after the administration of the dye. In each of these cases, normal blood iodines were obtained prior to cholecystography. In a number of patients, iodine values of 10 to 40 micrograms per cent were observed shortly after the use of tincture of iodine on the skin for clyses, infusions, and transfusions.

Severe degrees of jaundice produced in cats by ligation of the common bile duct were not accompanied by significant elevation of the iodine content of the blood.

The elevated blood iodine in patients with diseases of the liver and biliary tract reported by previous investigators may be explained primarily by the antecedent administration of iodine, particularly of the iodine-containing compounds used in cholecystography. No clinical or experimental evidence has been found to indicate that the liver exerts a specific function in regulating iodine metabolism.

The Relation of Hypertension to Coronary Atherosclerosis

By DAVID DAVIS and (by invitation) MAX J. KLAINER, Boston, Mass.

The relation of hypertension to coronary disease was investigated in 507 cases, 101 of these were studied by the injection-dissection technique of Schlesinger.

Patients with essential hypertension showed an appreciably higher incidence of severe coronary disease than controls in each decade. Below the age of 50 the differences were as marked, or more so, than in the higher age

On the other hand, a group of patients with severe hypertension associated with primary renal disease and finally uremia showed a lower incidence of severe coronary disease than controls in the same age groups.

The relation of the severity of hypertension to the degree of coronary atherosclerosis was further investigated in the patients with essential hypertension by considering both blood pressure levels and heart weights as indices of severity. Patients with severe hypertension showed the same incidence of coronary disease as those with mild hypertension. These findings (1) the low incidence of coronary disease in patients with renal hypertension, and (2) the lack of relationship between the degree of hypertension and the severity of coronary disease in essential hypertension, are evidences that the hypertension *per se* is not a factor in the production of coronary atherosclerosis.

Iron Deficiency and Anemia Associated with Carcinoma of the Proximal Portion of the Colon By R. L. CLARK, M. H. POWER, and FRANK J. HECK (by invitation), and E. G. WAKEFIELD, Rochester, Minn.

The type of anemia that occurs with carcinoma of the proximal portion of the colon has been observed to be the same as that produced by a deficient supply of iron for elaboration of hemoglobin. The abnormal demands made upon the iron supply of the body are increased in instances in which the lesion is situated in the proximal portion. Study of the concentration of iron in the serum has

furnished additional confirmation of a deficiency of iron. In all cases of cancer of the right half of the colon, the concentration of iron in the serum was low. If severe anemia was present, a very marked decrease of the concentration of iron occurred. The anemia apparently can be arrested if a sufficient amount is absorbed. Recovery from the anemia following removal of the cancer was demonstrated to be dependent upon adequate absorption of iron. Administration and absorption of iron following resection of the involved segment of the colon resulted in a return of the normal concentration of iron and hemoglobin in the serum. In two cases the anemia accompanying carcinoma of the distal portion of the colon has been observed to be similar to that occurring in conjunction with carcinoma of the proximal position of the colon.

Carbohydrate Metabolism in Paget's Disease By T. L. ALTHAUSEN and A. M. BASSETT (by invitation), San Francisco, Calif.

Decreased dextrose tolerance is commonly found in Paget's disease, and is usually ascribed to a diabetic tendency probably caused by a disturbance of the hypophysis.

Eighteen patients with Paget's disease were given our test for intestinal absorption consisting of oral administration of 40 grams of galactose followed by a blood galactose curve. This test gives normal results in diabetes mellitus. Among 18 patients with Paget's disease, increased intestinal absorption was found in 15. The average peak of the curve in the whole group was 57 mgm. per cent as compared to a normal peak of 19 mgm. per cent.

There was positive correlation between the galactose and dextrose tolerance curves. Impaired utilization of galactose was ruled out by intravenous administration of galactose. There was no correlation between the increase in blood phosphatase or the basal metabolic rate in Paget's disease and increased intestinal absorption of galactose.

Our data indicate that accelerated intestinal absorption rather than impaired utilization accounts for the decrease in sugar "tolerance" in Paget's disease.

Fractionation of the Serum Proteins and the Takata-Ara Reaction in Cirrhosis of the Liver By JAMES A. DAUPHINEE, W. R. CAMPBELL, and (by invitation) M. I. HANNA, Toronto, Can.

In certain forms of liver disease, particularly hepatic cirrhosis, there is a definite alteration in the level and character of the serum proteins. This alteration consists in a decrease in the total protein which is accounted for largely by a decrease in the serum albumin. However, in a number of instances of cirrhosis there is a decrease in the albumin-globulin ratio not only because of a decrease in the albumin but also because of an actual increase in the total globulin.

The Takata-Ara reaction has also been found to be positive in many cases of cirrhosis and to be negative in a wide variety of other conditions with relatively few exceptions. These exceptions are commonly associated with an increase in the globulin fraction of the serum proteins.

By the use of the rapid and convenient sodium sulphite method of Campbell and Hanna for the separation of the serum protein fractions the levels of serum albumin pseudoglobulin I pseudoglobulin II euglobulin and other proteins precipitated at a lower concentration than 15 per cent sodium sulphite have been investigated in cirrhosis of the liver and in other conditions. An attempt has been made to correlate these findings with the results of the Takata Ara reaction.

Many cases of cirrhosis of the liver show a definite elevation of the serum globulin which is largely accounted for by an increase in the euglobulin fraction. There is a close agreement between this elevation of the so-called euglobulin fraction and the positive Takata Ara reaction although the ratio of this material to the albumin portion may play some part. A peculiar feature noted in this series of cases is the frequency with which the Takata Ara reaction appears to be correlated with fractions of serum protein intermediate in their behavior to sodium sulphite between euglobulin and fibrinogen. In certain other conditions where there is a similar change in the serum protein fractions the same correlation seems to exist, but this is not consistently the case.

Variations in the Acid Base Balance of the Internal Jugular Blood of Man Induced by Voluntary Hyperpnea By L. F. NIMS (by invitation) New Haven Conn. and W. G. LENOX and F. A. GIBBS Boston Mass.

Samples of jugular blood were taken from 28 selected patients before during and after short periods of voluntary hyperpnea. From some of the patients simultaneous arterial samples were taken from the femoral artery. The carbon dioxide content the oxygen content and the oxygen capacity of the blood was measured by means of the Van Slyke method and apparatus. To define the acid base condition of the blood it is also necessary to determine either the pH or the carbon dioxide tension of the blood. In the present experiments the pH was determined with a glass electrode, and the carbon dioxide tension of the blood was calculated from the Henderson Hasselbach equation.

As is well known overventilation produces changes in the acid base balance of the blood characterized by a shift to more alkaline pH and a lowered carbon dioxide tension. This investigation adds somewhat to our knowledge of how these changes occur with time. The results also indicate that the brain has some control over the acid base balance of the jugular blood. During the hyperpnea, for example the pH change that occurs in the venous blood is only about half that which occurs in the arterial blood.

Immunological Observations of Patients with Pneumococcal Pneumonia Treated with Sulfapyridine By MAXWELL FINLAND and (by invitation) WILLIAM C. SPRING JR. and FRANCIS C. LOWELL Boston Mass.

The studies were carried out in the same manner as those by Finland and Brown in cases of Type III pneumonia. It was found that sulfapyridine exerts a greater bacteriostatic effect in whole blood *in vitro* on both Type I and Type III pneumonia in lower concentrations than sulfanila-

uide. In addition a definite bactericidal effect was noted for large numbers of pneumococci of these types. With sulfanilamide, bactericidal action was demonstrated only in occasional patients when large amounts of the drug were added. In patients under treatment with sulfapyridine, the bacteriostatic and bactericidal effect of the blood corresponded to the *in vitro* results obtained when corresponding concentrations of the drug were added to the blood obtained prior to treatment. Actively acquired antibodies (agglutinins and mouse protection) appeared at the usual time irrespective of the febrile course. The optimum results were obtained when specific serum was given in combination with sulfapyridine or in patients treated with the drug alone when they had pneumococcal activity or had evidence of the appearance of protective antibody at the time treatment was begun. In a number of cases of Type III pneumonia recovery occurred although no evidence of type-specific antibodies could be demonstrated after treatment with sulfapyridine was discontinued.

The Blood Volume in Bright's Disease and Hypertension By JOHN G. GIBSON 2d and ALFRED W. HARRIS (introduced by Henry A. Christian) Boston Mass.

Blood volume studies in patients with Bright's disease were made by the method of Gibson and Evans (J. Clin. Invest. 1937, 16, 301). Cases were grouped according to the clinical classification of Bright's disease of Christian (Interstate Postgraduate Assembly 1931, pp. 71 to 74).

In a group of 16 hypertensive patients without congestive heart failure there was no significant variation from the normal in plasma, circulating red cell, or total blood volume even in markedly plethoric appearing patients.

In 12 patients with chronic glomerular nephritis without edema and 10 patients with subacute glomerular nephritis with edema (nephrosis syndrome) total blood volume was definitely below normal due chiefly to a diminution in the circulating red cell volume. Plasma volume being normal or slightly above. As azotemia progressed plasma volume tended to increase and circulating red cell volume to diminish in a linear relationship to the decrease in the red cell count but total blood volume remained below normal. In the anemia of nephritis the percentage reduction below normal in circulating red cell volume bore the same relationship to the red blood cell count found in both primary and secondary anemia.

In the 5 cases with congestive heart failure plasma, circulating red cell and total blood volume while not as high as the level found in congestive failure in valvular or chronic myocardial disease (J. Clin. Invest. 1937, 16, 831) were definitely higher than the average volume values at comparable levels of anemia found in the group of nephritics without congestive heart failure. In one case cardiac compensation was accompanied by a marked decrease in plasma, circulating red cell and total blood volume even though the red cell count was about 2 million during failure and subsequently.

Crystalline Insulin By ALEXANDER MARBLE and (by invitation) ILMARI VARTIAINEN, Boston, Mass

Although "crystalline insulin" (Solution of Zinc-Insulin Crystals) was released for sale in August 1938, the rapidity of action and duration of effect of this preparation have remained controversial. Insulin of crystalline type has been regarded by many as possessing a prolonged action.

In comparative studies carried out in both normal and diabetic individuals and in normal rabbits, the crystalline and amorphous types of insulin caused a prompt fall of the blood sugar which was almost identical in rate. Both types, therefore, must be regarded as rapidly-acting.

In normal rabbits the duration of action of the crystalline type was almost identical with that of the amorphous variety. In normal and diabetic human subjects the action of the former type seemed slightly prolonged. With the diabetic individuals there occurred on the average a slightly greater blood-sugar-lowering effect with insulin of crystalline type. Following a single subcutaneous injection on a day during which food was withheld, the lowest point in the blood sugar curve was reached, on the average, in 6 hours in the case of the amorphous, and in 7 hours in that of the crystalline variety. In the return toward the initial value, there was a lag of approximately two hours when the crystalline type was used.

Differences in values for sugar in blood and urine of diabetic patients during days of maintenance first on one type and then on the other type of insulin, were so slight as to be of relatively minor importance clinically although with some patients slightly lower values were obtained with the crystalline variety.

To simplify treatment, it is advisable to decrease rather than increase the number of types of insulin on the market. At the present stage of development it seems desirable to limit these to (a) a rapidly-acting insulin which, if commercially practicable, might be of the amorphous type, and (b) a slowly acting variety, the protamine zinc insulin.

Periods of Crisis and of Stabilization in Addison's Disease

By JAMES A. GREENE and (by invitation) GEORGE JOHNSTON, Iowa City, Iowa

Balance studies of sodium, potassium, and chloride have been made in a patient with Addison's disease. At first, while sodium was being stored, crises occurred frequently, although large amounts of sodium and cortical extract were administered. Later, as a sodium balance was established the crises promptly ceased. Sodium balance was then maintained without administration of cortical extract, and even after the sodium intake had been reduced about one-third for 24 days. A negative sodium balance was then produced by a greater reduction of sodium intake and was maintained for 11 days during which approximately 37 grams of the stored sodium was excreted, but crises did not develop.

The study shows that the patient was strikingly less sensitive to omission of cortical extract or reduction of sodium intake after sodium equilibrium had been established than during the period of sodium storage. In addition, the period of induced negative sodium balance,

following that of sodium equilibrium, appears to be the best time for studying the effect of administration or omission of cortical extracts, or of alteration of sodium or potassium intake. The effect of orally and hypodermically administered cortical extract upon the storage of sodium is also reported.

The Association of Peptic Ulcer and Gallbladder Disease with Coronary Atherosclerosis: A Postmortem Study By BERNARD J. WALSH (by invitation), and EDWARD F. BLAND and PAUL D. WHITE, Boston, Mass

Because of recent interest in the combination of gallbladder and gastro intestinal lesions with coronary arterial disease produced in dogs by the injection of acetylcholine (Hall) we have thought it important to compare the incidence in man.

Clinical Experiences with a Synthetic Estrogen, Stilboestrol

By EPHRAIM SHORR and (by invitation) GEORGE N. PAPANICOLAOU, and BENJAMIN F. STIMMEL, New York, N. Y.

The recent synthesis by Dodds and coworkers of an estrogenic substance, Stilboestrol (4,4'-dihydroxy- α , β -diethyl stilbene) has aroused considerable interest because of its powerful estrogenic activity, orally, as well as by subcutaneous use, and its chemical structure which differs greatly from the naturally occurring estrogens. Recent clinical trials in England have demonstrated its estrogenic activity in the human, its ability to produce the customary vaginal and endometrial changes, and the attendant relief of menopausal symptoms. Its low cost and oral effectiveness should make it a valuable therapeutic agent providing its use is without harmful features. The reports from England have mentioned an occasional transitory nausea by oral route, which was absent by subcutaneous injection.

Our experience with the use of this drug is as follows. Its estrogenic character and ability to relieve symptoms is confirmed. A large percentage of the patients, however, have experienced severe gastro intestinal disturbance not only by the oral route but intramuscularly. This effect of the drug appears to be central in origin. Until its significance is known, great caution seems to be warranted in the use of this drug to replace the natural estrogens.

The Effect of Anterior Pituitary Injections on the Blood Acetone Bodies of Adrenalectomized Rats By REGINALD A. SHIPLEY (introduced by Joseph T. Wearn), Cleveland, Ohio

Fasting adrenalectomized rats have been tested for their blood acetone body response after injections of crude anterior pituitary extract by the analyses of samples of tail blood taken before and after injection. Graduated doses of extract were given to groups of both adrenalectomized and normal rats in order that the respective assay curves could be compared. The adrenalectomized rats were sensitive to the extract but their response was only one half to one-third that of unoperated rats when a comparison was made on the basis of the size of dose necessary to produce a given response. These findings indicate that

the ketogenic activity of the pituitary gland is not necessarily mediated through the adrenal cortex.

Simultaneous blood and urine determinations were made in adrenalectomized rats receiving anterior pituitary extract. The results suggest that ketonuria fails to occur in these animals because the rise in blood acetone bodies is sufficient to exceed the urinary threshold.

Studies of Epidemics of Influenza which Occurred in 1939

By FRANK L. HORSFALL JR., and (by invitation)
MONROE D. EATON, RICHARD G. HAHN and ELMER R. RICKARD, New York, N. Y.

Clinical studies were made of 174 cases in which the symptomatic syndromes were typical of mild epidemic influenza. The cases occurred in three localized epidemics in New York State during January 1939. Throat washings were taken from 64 representative cases early in the disease and 32 of the washings have been inoculated intranasally in ferrets. Although aural passages were made in ferrets fever and nasal symptoms characteristic of epidemic influenza virus infection were observed in only 10 or 31 per cent, of the passage series. The difficulty that has been encountered in the isolation of the etiological agent from these cases of epidemic influenza contrasts sharply with the fact that serological diagnoses were made with ease. Studies of the acute and convalescent serum from the same cases have indicated that all but two did have epidemic influenza virus infection. Cross immunity tests in ferrets with two of the strains of influenza virus isolated from these epidemics have shown them to be antigenically different from strains isolated in previous years.

The Glucose Supply to Joint Cartilage By ERIC G. L. BYWATERS (by invitation) and WALTER BAUER, Boston Mass.

The glucose which joint cartilage has been shown (by manometric methods) to use reaches it by diffusion from synovial fluid. If the concentration here falls glucose deficiency will develop in the deeper layers of cartilage.

We have therefore investigated the entrance of glucose and other crystalloids into the joint cavity following intravenous injection and its removal therefrom, both in normal calves and in patients with knee effusions.

It has been shown that whereas in normal joints there is a prompt rise to above venous level (as has been demonstrated in other body fluids) in certain diseased joints there is a very small slow and ill-sustained rise reaching a delayed peak at one-fifth of the blood level. This is associated with a consistently low resting glucose level in the synovial fluid and with advanced pathological changes in the joint.

That this failure to reach blood level is due to utilization can be shown by the injection of glucose into these joints. The level rapidly falls to well below blood level and utilization can thus be measured. From a knowledge of the metabolic activity of cartilage, leukocytes and synovial membrane it can be shown that this sugar removal is due in an amount of synovial membrane comparable to that removed at synovectomy in similar cases.

Glucose deficiency may thus play some part in the im-

pairment of cartilage seen in advanced rheumatoid arthritis and hence synovial sugar determinations may indicate the proper time for the operative removal of this inflammatory tissue.

The Gravimetric Determination of Serum Proteins By BERNARD M. JACOBSON, Boston Mass.

In a few pathological sera there were noted discrepancies between the values for protein content afforded by gravimetric determination and the results of the commonly used method of nitrogen determination. These observations led to a systematic study of both normal and pathological sera. Total nitrogen was determined by the Kjeldahl procedure, the protein nitrogen was multiplied by the customary factor 6.25. The gravimetric determination was carried out by quantitatively precipitating the serum proteins with acetone, washing the precipitate with acetone and with ether drying to constant weight and finally ashing. This acetone precipitate was uncontaminated with lipoids or with significant amounts of non-protein nitrogenous substances. Precipitation of serum with trichloroacetic acid followed by washing of the precipitate with acetone yielded protein values identical with those obtained by direct precipitation with acetone. On the other hand considerably lower values were obtained by heat coagulation of the sera, for filtrates of such coagula contained much non-coagulable protein brought down by either acetone or trichloroacetic acid.

In a large number of sera the total protein content was determined both gravimetrically and by means of the Kjeldahl method. In every instance the protein content obtained gravimetrically exceeded the value afforded by the nitrogen determination. This difference ranged from 0.11 to 0.72 gram of protein per 100 cc. of serum or from 1.6 to 13.5 per cent of the Kjeldahl value. In four fifths of all instances the difference exceeded 4 per cent of the Kjeldahl value.

The explanation of these discrepancies was furnished by the results of direct determination of the nitrogen content of the acetone precipitates. On an ash free basis such precipitates contained from 14 to 15 per cent nitrogen instead of the value of 16 per cent implied by the usual conversion factor of 6.25. However the use of any other factor for all sera was found inaccurate consequent upon the variable nitrogen content of the total proteins of different sera. Thus for more exact determination of serum proteins the gravimetric method is recommended. The labor required by the gravimetric procedure is no greater than that involved in the Kjeldahl determination.

Peripheral Resistance and Vascular Reactions in Arterial Hypertension By EUGENE A. STEAD JR. and PAUL KUNKEL (introduced by Soma Weiss) Boston Mass.

The nature of the arteriolar resistance and its distribution in the body remain the fundamental problems in the study of the etiology of arterial hypertension. Since the cardiac output is normal in this disease, the average arteriolar resistance at rest must be increased. It is essential however to obtain measurements on the specific state of the arteriolar resistance in various organs and tissues and

to determine whether the peripheral resistance in any one organ can be reduced to normal by physiological vasodilating stimuli. Evidence that the peripheral resistance is uniformly high throughout the various organs and tissues and cannot be reduced to normal by physiological stimuli has been obtained by means of (1) plethysmographic determinations of the blood flow in the foot and hand, with the vessels widely dilated by local heat of 43° C, (2) plethysmographic determinations of the blood flow in the muscles of the forearm, with the vessels widely dilated by exercise, (3) indirect estimations of the blood flow through the brain by arteriovenous differences, (4) indirect estimations of the peripheral resistance in the brain by postural experiments after the administration of sodium nitrite. There was no significant difference in blood flow in any of the above organs between normal and hypertensive subjects. Therefore, as the pressure head is greater in hypertensive subjects, the peripheral resistance in hypertension must be uniformly increased in each of these parts, otherwise the blood flow would be greater than normal. Moreover, the finding of a constant increase in the arteriolar resistance in the skin, muscle, and brain in the presence of a normal cardiac output suggests a similar increase in vascular resistance in the abdominal viscera.

In two subjects with marked hypertension, the blood pressure was greatly reduced for a time by a course of malarial therapy. In one of these subjects, in whom hypertension had been known to be present for over 4 years, the blood flow in the dilated foot decreased when the blood pressure was lowered, indicating that the peripheral resistance was still high, even in the absence of hypertension. In the second subject, who was known to have had a normal blood pressure 2 years previously, the blood flow in the dilated foot did not change when the blood pressure was lowered, indicating that with the fall in blood pressure the peripheral resistance had become normal. Both of these subjects were afebrile and ambulatory when the blood flow determinations were made.

The vasomotor reactions in the hand and foot were similar in normal and in hypertensive subjects. A marked fall in pressure in two hypertensive subjects after malarial therapy caused no change in the vasomotor reactions. Postural studies and plethysmographic measurements of venous tone indicated that the response of the veins to sodium nitrite in normal and in hypertensive subjects was the same.

Further Clinical and Experimental Studies on the Ballistocardiogram By ISAAC STARR, Philadelphia, Pa

In the 1938 presentation emphasis was placed on the amplitude of the ballistic curves, which are a function of cardiac output. Since then we have been giving attention to the interpretation of the abnormalities in the form of the records which have been found in certain cases.

Impacts similar to those seen in disease may be derived theoretically by assuming abnormal curves of blood velocity in the aorta and pulmonary artery during a single systole.

Similar abnormal types of ballistic curves have been

reproduced in animal experiments by asphyxia, by chloroform, and by directly damaging one side of the heart when the other was left intact.

Therefore it is concluded that the shape of the ballistic record is determined by the changes of blood velocity in the aorta during systole. When the heart is normal, maximum blood velocity is attained early in systole, when the heart is diseased, maximum velocity is attained late in systole. These conditions can be diagnosed by the ballistocardiogram.

If one side of the heart is weak, and the other strong, the ballistocardiogram is also characteristic.

The Functional Measurement of the Number of Active Glomeruli and Tubules in the Kidneys of Normal and Hypertensive Subjects By H CHASIS, H A. RANGES, W GOLDRING, and H W SMITH (introduced by James A Shannon), New York, N Y

In 1924 Richards and Schmidt (*Am J Physiol*, 1924, 71, 178) noted by direct microscopic observation that only a certain proportion of the glomeruli, or of the capillaries in individual glomeruli, were active at any one moment. This observation has been repeatedly confirmed, and it has been inferred from the behavior of glomeruli in the frog's kidney that there is similar intermittency of glomerular activity in the human kidney. Did such intermittency exist it would acquire increased significance in view of the possibility of a direct blood supply to the tubules in the human kidney via arterial-venous anastomoses (Spanner, *Ergänzungsheft z Anat Anz*, 1938, 85, 81). The physiological importance of this point, and the clinical importance of determining whether there exists any tissue in the hypertensive kidney which is rendered ischemic by reversible vasoconstriction of renal arterioles has prompted us to measure the quantity of active glomerular and tubular tissue by saturation methods.

The total active tubular tissue in the kidney has been measured by elevating the plasma level of diodrast to a point where all the tissue receiving blood is excreting this substance at the maximal rate (diodrast-Tm, as described by Smith, Goldring, and Chasis, *J Clin Invest*, 1938, 17, 263). The total active glomerular tissue has been measured by raising the plasma glucose to a point where the tubules are reabsorbing this substance at the maximal rate (glucose-Tm, as described by Shannon and Fisher, *Am J Physiol*, 1938, 122, 765). If any glomeruli can be opened or closed by physiological means the fact will be revealed by an increase or decrease in the number of nephrons reabsorbing glucose, and therefore by a corresponding change in the value of glucose Tm. At appropriate plasma levels of diodrast and glucose these measurements reveal the quantity of functioning tissue, and are independent of variations in renal blood flow or filtration rate.

Diodrast-Tm and glucose Tm have been measured in normal and hypertensive subjects under basal conditions, during renal ischemia induced by adrenin, neosynephrin, tyramine, etc., and during sustained renal hyperemia.

Variations in the Codehydrogenase I and II Content of the Blood and Urine in Health and Disease By RICHARD W. WILTER and SUE POTTER WILTER (by invitation), and TOM D. SPIES Cincinnati, Ohio

A method for the determination of codehydrogenases I and II sensitive to 1 part per 200 000 000 has been devised and used in this investigation. The codehydrogenase values found in the blood and urine of 30 well nourished members of the hospital staff were arbitrarily designated as normal. In contrast, the codehydrogenase in patients with pellagra in relapse, severe diabetic acidosis, lobar pneumonia, various malignant tumors, leukemia, and third degree burns was found to be 1/2 to 1/120th of normal values.

Repeated determinations were made before and during and after therapy with nicotinic acid, riboflavin or thiamin on samples of blood and urine from 10 pellagrins. The cozymase increased 400 per cent within 24 hours following the oral administration of nicotinic acid. Ten cases of lobar pneumonia showed 100 per cent increase in blood codehydrogenase within 6 to 24 hours after crises induced by serum or sulphapyridine. 10 cases of diabetic acidosis studied before and after control by recognized therapy with insulin fluids, and glucose showed codehydrogenase values comparable to those found in pellagrins. 10 cases of malignant tumors before and after x-ray therapy were studied. Before therapy the tumor cases were found to have codehydrogenase values 1000 per cent greater than those found in the blood of 15 patients suffering from leukemia, both lymphatic and myeloid who were followed to the hospital and at home for periods of time up to 5 months. The extremely low values for blood codehydrogenase found in the leukemic group of patients failed to respond to large doses of nicotinic acid. In contrast to the pellagrins but could be increased 12 000 per cent when yeast, nicotinic acid and riboflavin were administered. Values of blood codehydrogenase 1/100th of normal were found immediately following hospital admission, in three individuals with third degree burns.

Thus the variations of codehydrogenase in the human body during severe infection, metabolic disorders, malignant tumors, leukemia, and burns have been determined and have been compared to a group of 30 healthy well nourished individuals who were used as controls.

The significance of these results will be discussed.

Factors Influencing Edema Formation in the Eyelids of Man By GEORGE E. BURCH (introduced by J. H. MUSSER) New Orleans, La.

This presentation includes observations demonstrating marked distensibility of the tissues of the eyelids, greater in amount than that found for any other common edema site studied. Although the subcutaneous tissue pressure of the lower lids normally is about equal to that of the forearm, the loose areolar tissue and markedly stretchable skin renders the eyelids extremely distensible, to such an extent that with the accumulation of interstitial fluid they cannot benefit by the limiting influences of the skin and tissue pressure in curtailing edema formation. The linear rate of lymph flow to the superficial lid lymphatics was found to be

greater with lid movement, blinking, and with the subject in the upright or sitting position than in the relaxed supine position.

This study was conducted on 80 living individuals: 50 normal and 30 with edema of the eyelids due to various causes. The subjects varied from 8 to 84 years in age and included males and females of the white and negro races in about equal numbers.

The mean subcutaneous tissue pressure measured by a method previously described for 21 normal lower eyelids was found to be 23.4 ± 0.7 mm. of water with a standard deviation of 5.1 ± 0.5 and minimum and maximum values of 16 and 32 respectively. For short periods of time the tissue pressure in the lids was not significantly affected by variations with respect to heart level or by loose closure of the eyes as in sleep. Subcutaneous injections of 1 cc. of normal saline (NaCl) into 10 lower lids produced practically no rise in tissue pressure, a moderate but significant rise in 9 atrophic breasts and 9 loose abdominal walls of multiparae and 8 prepuces, and a marked rise in the volar surfaces of 10 forearms and 10 pretibial areas. In 30 patients with various types of edema of the eyelids the tissue pressure was found to be only slightly elevated; the pressure in the lids, in spite of the edema, being lower than that in remote non-edematous areas as the forearm and prepuce. The linear rate of lymph flow in the superficial lid lymphatics measured by the McMaster method of 50 eyelids in 20 normal subjects was increased over that in the relaxed supine position by sitting and by lid movement, blinking. The skin distensibility ("stretchability"), determined by a method previously described of the 20 lower lids of 10 normal subjects, was found to be 1.23 ± 0.06 mm. per cm. of skin per 20 grams of force with a standard deviation of 0.43 ± 0.05 . The skin of the lids was found to be more distensible than the skin of any area studied, being approximately 3 times as distensible as that of the skin of the pretibial area, 2.5 times that of the volar surface of the forearm, twice that of the dorsum of the hand, 1.75 times that of the dorsum of the foot, and 1.2 times that of the abdomen.

The interplay of these factors favors the development of edema in the eyelids and in systemic diseases equally affecting the interchange of fluid between the blood vessels and tissue spaces generally, as in acute hemorrhagic nephritis, the eyelids would be prone to an early development of edema, especially at night, with a tendency to recede during the day with activity.

Further Evidence for the Role of the Adrenal Cortex in Carbohydrate Metabolism: Relation of the Adrenal Cortex to Amylase Activity in Blood Serum and Liver of the Dog and Rabbit By OLIVER COPE and (by invitation) ISRAEL KAPNICK, ADRIAN LAMBERT, T. DENNIE PRATT and MAX G. VERLOT, Boston, Mass.

In the adrenalectomized dog upon withdrawal of cortical extract, there is a sharp rise in amylase activity of the blood serum. The level of amylase activity reached in 24 hours, although not maximum, is equal to that seen in the hypophysectomized dog or in human pancreatitis. With the development of adrenal insufficiency there is a

further progressive moderate rise in activity until death. The final level reached is higher than that we have observed under any other condition. This rise in blood serum amylase activity is the most sensitive objective measure of adrenal cortical insufficiency yet observed in the dog. Following the removal of only one adrenal gland in four of seven dogs there was a rise in serum amylase activity returning to normal within a few weeks. After the removal of the second adrenal and the withdrawal of cortical extract, in all dogs the high level of serum amylase activity is reached 24 hours or longer before any changes are demonstrable in the hematocrit, nonprotein nitrogen, serum protein, serum sodium, potassium, or chloride. Doses of adrenal cortical extract (Eschatin) sufficient for maintenance of the adrenalectomized dog in apparent normal state, as judged by vigor, appetite, and the above laboratory data other than the amylase, are not sufficient to maintain the serum amylase activity within normal limits. Five to ten times the dose needed for ordinary maintenance is required to keep the serum amylase activity within normal limits after removal of the second adrenal or to return and maintain it within normal limits after cortical insufficiency has been allowed to develop.

The amylase activity of the liver in terminal insufficiency is increased over the normal. It is possible but not proven that the changes in the serum amylase reflect changes in liver function.

In the adrenalectomized rabbit the changes in the blood amylase activity are the reverse of those seen in the dog. The changes, however, are of a lower order.

In spite of the sensitivity of the amylase system of the dog to changes in adrenal cortical function it is not concluded that control of the amylase system by the cortex is the primary function of the gland. The difference in the behavior between the dog and the rabbit suggests that there may be some intermediary step as yet unsolved.

Effect of Orally Administered Cortical Extract upon Sodium and Chloride Balance in Addison's Disease By JEROME W. CONN and FREIDA W. SILVERMAN (introduced by L. H. Newburgh), Ann Arbor, Mich.

Several reports indicate that adrenal cortical extract is effective when administered orally to adrenalectomized animals. We know of only one study (Thorne, 1938) in which the effect of oral administration of cortical extract in Addison's disease has been evaluated by means of electrolyte balance. Thorne found that $2\frac{1}{2}$ times more extract was necessary by mouth than when injected.

In the present investigation, a severe case of Addison's disease was fed a constant diet for 55 consecutive days. The diet was maintenance in calories and contained 7.5 grams of sodium salts. Water intake was constant. The comparative effectiveness of parenterally and orally administered cortical extract, based upon sodium and chloride balance in addition to clinical manifestations were thus observed.

Conclusions

1 Adrenal cortical extract is physiologically effective when taken orally.

2 In terms of fresh gland, half as much extract was needed by mouth as when given parenterally to bring about sodium and chloride equilibrium.

3 When 7 grams of sodium chloride were added to the basic diet the amount of orally administered extract needed to maintain sodium and chloride balance was half of that required on the basic diet alone.

These results are in accord with reported observations on adrenalectomized animals.

Experimental Studies on Headache: Observations on Pain Pathways By GEORGE A. SCHUMACHER (by invitation) and HAROLD G. WOLFF, New York, N. Y.

The purpose of the investigation was to determine what afferent nerves conduct the impulses interpreted as headache. Headaches were induced experimentally by injecting 0.1 mgm. of histamine phosphate intravenously. Normal subjects regularly develop bilateral headache under these circumstances. Patients who had partial or complete sections of the sensory root of the trigeminal nerve, with partial or complete hemi-analgesia of the face and anterior half of the scalp were investigated (operations performed by Dr. Bronson S. Ray). Also studied were patients who had sections of the upper cervical sensory roots with resultant occipital hemi-analgesia. Patients who had dorsal root or brain stem disease resulting in partial or complete analgesia on one side of the back of the head were likewise investigated.

It has been shown that histamine headache results from the stretch of cranial arteries. In order to be sure that the cranial arteries were being stretched adequately during these experiments photographic records of cranial artery pulsations were made during each induced headache.

Four patients who, as a result of incomplete section of the trigeminal sensory nerve root, had unilateral loss of sensation over the lower part of the face, had headache induced by histamine on both sides of the head, in the front and back.

Five patients who, as a result of complete section of the trigeminal sensory nerve root, had, in addition to hemi-analgesia of the lower half of the face, unilateral loss of sensation over the frontal, temporal, and parietal areas, did not have headache induced by histamine in these regions. However, they had headache elsewhere in the head, including the opposite fronto-parietal region and the back of the head.

The ligation of the middle meningeal and temporal arteries (necessary for trigeminal root section) did not explain the absence of "induced" headache. This was shown by the occurrence of the headache when the arteries were ligated as above, but the trigeminal root was only partially transected.

Two patients who had unilateral loss of sensation in the occipital region did not have headaches induced in this region by histamine. They did, however, have headaches elsewhere in the head, including the opposite occipital region and the front of the head.

Other data indicated that there were additional, though less important afferent pathways. In short, impulses from cranial arteries of the front of the head conducted through the sensory root of the fifth cranial nerve, were mainly

responsible for frontal temporal and parietal headache. The upper cervical sensory roots conveying impulses from cranial arteries from the back of the head were chiefly responsible for occipital headache.

The Effects of Heated Heterologous Kidney Extracts on Blood Pressure By EUGENE M. LANDIS and (by invitation) W. A. JEFFERS, Philadelphia, Pa.

The injection of simple saline extracts of kidney tissue both homologous and heterologous, is frequently followed by unpredictable results ranging from sudden death of the injected animal to various combinations of pressor and depressor effects. Heated (55 to 56° C. for 20 minutes) and filtered extracts prepared from rabbits' kidneys consistently elevated the blood pressure of unanesthetized rabbits and did not contain depressor or lethal substances (J. Clin. Invest. 1938 17 189).

This method was used to prepare similar 10 per cent extracts from the kidneys of rats, guinea pigs, rabbits, dogs, and man. The solutions obtained were entirely clear yellowish or pink in color and contained approximately 0.10 per cent albumin and 0.12 per cent globulin. These extracts were preserved in ampules by freezing and desiccation *in vacuo* (Cryochem process) later they were redissolved, centrifuged and immediately injected into unanesthetized rabbits and into rats, guinea pigs, rabbits, and dogs under light nembutal anesthesia. Dosage was adjusted so that the animals received equivalent amounts of fluid and of solid extract according to body weight.

In this group of animals the pressor effects of the respective extracts were not species-specific, since each extract raised the blood pressure to some degree in all 4 species. However the potency of the extracts differed according to species in that extracts of rabbits' kidneys were most highly active throughout while those of human kidneys were often almost inactive, with rat and guinea pig extracts in an intermediate position as to potency. The average sensitivity of the 4 species also differed in that the guinea pig was most responsive, and the rabbit least responsive with rat and dog intermediate.

The results obtained with fractional ammonium sulphate precipitation followed by suitable dialysis indicate that heating to 55° C. while diminishing depressor and toxic effects, also precipitates or destroys at least some of the pressor substance, particularly in the extracts from kidneys of dog and man.

A Double Alternating Pressure Chamber to Provide Adequate Lung Ventilation Without Discernible Lung Movement.
By ALVAN L. BARACH, New York, N. Y.

Instead of a rhythmical increase and decrease in the volume of the lungs, which characterizes normal respiration, Thunberg (1926) suggested that a change of one-sixth of the barometric pressure applied to an individual within a chamber would result in adequate pulmonary ventilation without movement of the chest wall. The principle depends on the physical law that the number of gas molecules present in a gas container varies with the

pressure under which the gas is kept in the container provided the volume and pressure remain constant.

We constructed a small room in which an alternating pressure of 110 mm Hg was produced 25 times a minute using a large air compressor and a special valve mechanism. Animal and clinical studies showed that pressure in the positive cycle was applied to the outer chest wall earlier and was of slightly larger extent than that which was transmitted through the bronchi and alveoli to the inside surface of the chest. Continuous cessation of discernible lung movement was accomplished by placing the body of the patient in an inner chamber with the head protruding. Pressure to the outer chest wall was then delayed until the lungs were suitably filled with air. Under these circumstances no discernible chest movement took place while the lungs were being ventilated. The accomplishment of continuous lung rest has been shown to be clinically feasible and is accompanied by no appreciable alteration in the oxygen or CO₂ content of the arterial blood, the venous pressure or the circulation time.

A Common and Important Error in the Measurement of Auriculoventricular Conduction (P-R Interval) By PAUL D. WHITE and (by invitation) STEPHEN A. FOOTE and C. EDWARD LEACH, Boston, Mass.

In the course of routine electrocardiography we have discovered that an error is sometimes possible in the measurement of the P-R(Q) interval and therefore in the estimation of the auriculoventricular conduction time, which may result in an erroneous diagnosis of partial heart block with the clinical implications that attend such a diagnosis.

It has been the custom almost universally, to measure the P-R(Q) interval in Lead 2 and usually to consider the longest P-R(Q) interval as the correct P-R interval measurement. This has probably been done because the P waves and often the QRS waves are larger and better marked in Lead 2 than in the other leads. However an error can arise when the QRS in Lead 2 begins with an isoelectric phase which can be discovered only by the comparison of the QRS waves in the three leads.

Thus the Q wave in Lead 1 may neutralize an R wave in Lead 3 when these waves are of the same amplitude, time, and duration or they may partially neutralize each other thus erroneously adding to the P-R(Q) interval in Lead 2. Also R in Lead 1 and Q in Lead 3 a still more common combination may considerably neutralize each other to produce an isoelectric level in Lead 2.

It is important to bear in mind the possibility of this error and to measure the P-R intervals in Leads 1 and 3 as well as in Lead 2. Actually the shortest P-R interval is the correct P-R interval no matter what lead we study provided the P wave begins simultaneously in the three leads. We suggest that if the interval from the beginning of the P to the end of the S is found equal in the three leads the shortest P-R(Q) interval should be taken as the correct measurement of auriculoventricular conduction time.

The Clot Promoting Activity in Hemophilia of Berkefelded Normal Human Plasma Free from Fibrinogen and Prothrombin By EUGENE L. LOZNER, ROBERT KAREK, and F. H. L. TAYLOR (introduced by George R. Minot), Boston, Mass

Previous investigations have shown that platelet free normal human plasma is effective in accelerating clot formation of hemophilic blood both *in vivo* and *in vitro*. The activity of such plasma has been shown to be associated with the globulin fraction of the plasma proteins. Among the known constituents of this globulin fraction are fibrinogen and prothrombin. Using precipitation and adsorption methods it has been possible to make preparations from citrated normal plasma free from these proteins. After the removal of either prothrombin or both prothrombin and fibrinogen from citrated normal human plasma it was found that the remaining fluid promoted the clotting of hemophilic blood *in vitro*. When similar preparations were injected intravenously into patients with hemophilia, the coagulation time of their blood approached normal limits. Repeated injections maintained the shortened coagulation time of the patient's blood. The effects of the injections were entirely similar to those of unmodified normal human plasma or whole citrated blood.

The Significance of Changes in Synovial Fluid Mucin in Joint Disease By MARIAN W. ROPES (introduced by Granville A. Bennett), Boston, Mass

Knowledge of the characteristics and functions of mucin is essential for an understanding of the physiology of joints and determination of the origin and removal of mucin.

Synovial fluid mucin is composed of protein and polysaccharide elements the structure and mode of combination of which are not established definitely. The physicochemical properties indicate some functions of mucin. The high viscosity and resulting lubricating power of synovial fluid are due to mucin. The high base binding power of mucin explains the observed effect on distribution of calcium between plasma and fluid. Osmotic pressure studies indicate the significance of mucin in the exchange of water.

Changes in mucin in pathological fluids are of diagnostic value. In traumatic fluids the unit concentration is normal (0.85 gram per 100 cc). In fluids from specific infectious and rheumatoid arthritis the unit concentration decreases in accord with the degree of inflammation. Despite the decreased mucin concentration the unit concentration of glucosamine remains high indicating mucin breakdown. The characteristic precipitation of mucin is lost and the viscosity of the fluid decreased in accord with the severity of the arthritis. The findings indicate that the entrance of mucin is increased in inflammation and that the destruction of mucin is increased in severe infectious joints.

The similarity of these changes to those produced by "mucinase" (isolated from *B. Welchii*) suggests an enzymatic nature of the changes in pathological fluids. No mucinase has as yet been demonstrated in pathological fluids.

The Mechanism of Uric Acid Elimination by the Kidney By FREDERICK S. COOMBS (by invitation) and JOHN H. TALBOTT, Boston, Mass

Simultaneous inulin, creatinine, and uric acid clearances were done in normals and in 20 patients with gout. The patients were divided arbitrarily into three groups: (1) no disturbance of inulin clearance, (2) mild disturbance, (3) severe disturbance. Subcutaneous and osseous tophi were observed in subjects of each group.

Previous work has shown that inulin excretion may be taken as a measure of glomerular filtration. It is assumed that in man, uric acid is excreted in the glomerular filtrate and is not a product of tubular activity. Our data indicate that normals and gouty patients of the first group have a uric acid clearance of approximately 10 per cent of the inulin clearance. This means that 90 per cent of the uric acid is reabsorbed from the glomerular filtrate. Patients with a moderate disturbance of inulin and creatinine clearance have a similar uric acid clearance and tubular reabsorption. A progressive decrease in the excretion of phenolsulphonephthalein and the ability to concentrate urine above 1:020 is noted, however.

The third group shows severe impairment of inulin and creatinine clearance, phenolsulphonephthalein excretion, and ability to concentrate urine. Tubular reabsorption of uric acid is about 70 per cent. An approximately normal uric acid clearance results in spite of diminished glomerular activity. In this group only are nitrogenous products retained in the serum and the blood pressure elevated. Advanced glomerular and tubular dysfunction is presumed.

Clearance studies were repeated in several patients following the administration of cinchophen, colchicine, and salyrgan. No effect upon the inulin and creatinine clearance was noted. Diminished tubular reabsorption and increase in uric acid clearance was observed in patients without severe renal impairment following the administration of 45 grains of cinchophen or 2 cc of salyrgan. Patients with advanced gouty nephritis show minimal changes in uric acid clearance. Colchicine in therapeutic amounts produced no change in uric acid clearance.

It is concluded that gouty patients show no selective inferiority for excretion of uric acid. In advanced gouty nephritis a profound disturbance of glomerular filtration is observed without concomitant diminution of uric acid excretion. This is thought to be due to failure of reabsorption in the renal tubule. Cinchophen and salyrgan, similarly, prevent uric acid reabsorption in the tubule. The pathogenesis of the increased concentration of uric acid in body fluids of patients with gout is believed, therefore, to be a function of increased formation or decreased destruction and not impaired elimination.

Hypoaminoacidemic Crises in Young Children with the Nephrotic Syndrome By LEE E. FARR and (by invitation) DOUGLAS A. MACFADYEN, New York, N. Y.

In young children with the nephrotic syndrome, the incidence of acute febrile episodes with peritoneal symptoms was markedly increased in that group with plasma albumin below one gram per 100 cc contrasted with nephrotic children having a higher plasma albumin con-

centration During these acute episodes there was a very rapid fall in the plasma albumin concentration with a rapid rise to its previous level after recovery This change was independent of the proteinuria Neither the apparent clinical severity of the illness nor the effect on the plasma albumin level was directly related to the presence of blood stream or peritoneal infection A characteristic pattern was always followed to onset and recovery These facts pointed to a disturbance of protein metabolism Using the specific determination for amino acids developed by Dillon and Van Slyke which was adapted for blood by MacFadyen and Van Slyke, we have followed the cell and plasma amino acids of five children with the nephrotic syndrome over a period of months This work revealed a hitherto unknown disturbance of plasma amino acids which was a prolonged lowering of their concentration with occasional periods of critical fall and rapid recovery to pre-existing levels The crises occur independent of infection The acute disturbances were very closely correlated to the onset of and recovery from, severe and typical clinical manifestations These were identical with those noted in the acute illness usually accompanied by fatal pneumococcal peritonitis so often observed in these children Recovery from the nephrotic syndrome was accompanied by a gradual return of the plasma amino acid levels to a normal value We have termed the acute episodes hypoparminoacidemic crises

Ultrafiltrate Magnesium Studies in Hyperthyroidism By L J SOFFER and (by invitation) D A DANTES E B GROSSMAN and H H SOBOTKA, New York, N Y

The following report concerns itself with the study of magnesium metabolism in clinical and experimental hyperthyroidism The total and ultrafiltrable blood magnesium was determined in 20 normal individuals It was found that the percentage of the total magnesium bound presumably to proteins, varied from 31 to 22.1 per cent In 5 patients with neurocirculatory asthenia the percentage of bound magnesium varied from 9.1 to 20.6 per cent In 30 patients with hyperthyroidism where the basal metabolic rate varied from 30 to 106 the percentage of bound magnesium varied from 21.5 to 60.0 per cent It is evident, therefore that in hyperthyroidism there occurs an increase in the amount of circulating magnesium which is bound The increase in the bound magnesium occurs at the expense of the ionized form since the total blood magnesium remains unaltered in hyperthyroidism

There is apparently no relationship between the amount of bound magnesium and the level of the basal metabolic rate.

In 12 patients the bound magnesium in the blood was determined before and after the administration of iodine Before the administration of iodine the non filtrable magnesium varied between 21.5 and 49.8 per cent of the total while after administration the percentage of bound magnesium varied from 6.0 to 34.5 per cent.

In 11 patients similar determinations were made before and after operation Whereas before operation the percentage of bound magnesium varied between 26.0 and 49.8 per cent, after operation it varied between 0 and

23.0 per cent—a return to a perfectly normal value In 10 instances studies were conducted before and after the administration of iodine and after operation It was found that after the administration of iodine, there occurred some drop in the amount of bound magnesium which further dropped to normal levels after operation

The next step was to determine how the magnesium was bound From 50 to 200 mgm of thyroglobulin was injected intravenously in one dose into 5 dogs Total and ultrafiltrable blood magnesium was determined at intervals of 15 minutes 1.5 and 24 hours In each instance the increase in bound magnesium varied between 75 and 100 per cent over the control level This increase occurred within 1 to 5 hours after the injection The injection of equivalent doses of thyroxin and horse serum produced no change in the percentage of bound magnesium This would suggest that the thyroglobulin plays some part in binding the ionized magnesium

Studies of Cutaneous Capillary Blood Pressure in Man By L W EICHNA (by invitation) and JAMES BORDLEY III, Baltimore Md

A critical study has been made of two methods for determining human capillary blood pressure (1) the indirect pressure-capsule method of Danzer and Hooker and (2) the direct microinjection method of Landis To test the accuracy of these methods the capillary pressure in the nail fold was determined at various subdiastolic levels of venous pressure

In each of 30 capillaries studied by the direct method when the venous pressure in the arm was raised the capillary pressure promptly rose to exceed the increased venous pressure This was observed in 18 subjects with normal high and low arterial pressure.

In over 200 capillaries studied by the indirect method no significant rise in the capillary pressure reading was observed when the venous pressure was raised For example, with venous pressures as high as 50 mm. Hg capillary pressure was frequently recorded as low as 10 mm. Hg

In 9 experiments in which a single capillary was studied by both methods the same discrepancy was noted the directly determined capillary pressure always exceeded venous pressure while the indirectly determined pressure showed no correlation with venous pressure

That the capillary pressure actually rises to exceed venous pressure is indicated by the fact that in all experiments capillary blood flow continued in the presence of increased venous pressure It is believed therefore that the direct method gives accurate results the indirect method inaccurate results.

Bacterial Endocarditis (Acute and Subacute) Superimposed on Syphilitic Aortic Valvulitis By ALBERT L. BRAUNSTEIN and STUART R. TOWNSEND (Introduced by John T King Jr) Baltimore Md

Though syphilitic aortic valvulitis and vegetative bacterial endocarditis are well established separate clinicopathological entities, the concomitant occurrence of the two processes on the same valve has been regarded as extremely rare and very little has been known on this

subject To our knowledge only 11 proved cases have been reported We have had occasion to study several such cases at postmortem examination A thorough search through our autopsy protocols has revealed, moreover, that among 4936 routine autopsies there have occurred 9 cases in which bacterial endocarditis was primarily engrafted on previously syphilitic aortic valves This represents 15.5 per cent of all our cases of bacterial endocarditis (58 cases) and an incidence of 3.37 per cent in all our cases with syphilitic aortic valvulitis (267 cases)

An analysis of the clinical and pathological data of our own cases, as well as those found in the literature, reveals that both acute and subacute types of endocarditis are superimposed on syphilitic aortic valves (6 acute and 14 subacute) Clinically, in the *subacute cases* the only constant findings suggestive of bacterial endocarditis were progressive anemia and daily intermittent fever The occurrence of chills, chilly sensations, subjective sense of fever, petechiae, embolic phenomena, and nephritis was markedly reduced The predominating signs and symptoms were those referable to syphilitic aortic insufficiency with myocardial failure At autopsy the vegetations were small and were located on the ventricular surfaces of the valve cusps and *not along the occlusal margins* There was also a great tendency toward healing of the vegetations No evidence of rheumatism was present in any case In 4 cases *Streptococcus viridans* was recovered either antemortem or postmortem Blood cultures were not taken in most of the instances because the diagnosis of bacterial endocarditis was not suspected clinically

In the *acute instances*, evidences of septicemia were usually obvious and myocardial failure was also quite striking At autopsy there were no distinctive findings

From our findings we conclude that with obvious signs of bacterial endocarditis in the presence of syphilitic aortic insufficiency the bacterial process most likely exists on a valve other than the aortic However, one may suspect the presence of bacterial endocarditis on a syphilitic aortic valve when, in the presence of aortic insufficiency, there exist a gradually progressive anemia and daily intermittent temperature rises which cannot be explained by any other findings

Simultaneous Electrograms and Mechanograms from the Intact Human Subject By CARL A JOHNSON and GRANT LAING (introduced by J A Capps), Chicago, Ill

By a special method the authors have been able to take simultaneous mechanograms and electrograms from the esophagus and stomach of normal unanesthetized intact human subjects The mechanograms showed the changes due to the contractions of the organ under observation The electrograms showed changes due to the action of the heart as well as other changes which will be discussed The possible importance of the electrical changes in relation to smooth muscle contractions as well as the possible importance of these changes to clinical medicine are discussed Results of other experiments in which changes in the conventional electrocardiogram were produced by

inflation of the stomach by means of a stomach balloon will be shown

The Effects of Adrenal Cortical Extract and Potassium on the Electrolyte Balance in Addison's Disease By K A KLINGHOFFER (by invitation) and P H LAVIETES, New Haven, Conn

The salt intake of a patient with Addison's disease was so limited that he was in slight negative balance Large doses of adrenal cortical extract repeatedly converted the negative balance to a positive one, the effect persisting for less than 48 hours No other result of the administration of the extract was consistently observed Lowering the potassium of the diet had no demonstrable effect, nor did the subsequent increase to normal

The Fermentation Stimulating Effect of Vitamin B₁ and Related Substances in the Urine of Human Subjects By J ALLEN KENNEDY and HELEN FRANK (by invitation), and JOHN B YOUNG, Nashville, Tenn

The amount of vitamin B₁ and related substances excreted in the urine as determined by the stimulation of yeast fermentation is reported for a series of normal subjects and patients suspected of vitamin B₁ deficiency The possible significance of substances causing acceleration of fermentation other than thiamin, as degradation products of B₁, and a measure of vitamin B₁ metabolism *in vivo* is discussed

Subleukemic Splenic Reticulosis By C H WATKINS and H Z GIFFIN, Rochester, Minn

In 1934 we reported two cases of subleukemic splenic reticulosis, emphasizing the close similarity of this condition to splenic anemia Since that time we have seen six similar cases These patients have been treated conservatively by roentgen therapy with control of the condition in four of the cases A summary of the clinical findings, differential diagnosis, and treatment is given

Variations in the Serum Cholesterol Following Pneumonia By KENNETH B TURNER and (by invitation) ALFRED STEINER, New York, N Y

The relative stability of the serum cholesterol level for the individual has been established Acute infection is known to produce a hypocholesterolemia, but the behavior of the serum cholesterol in the convalescent period is less well known

In the present study the serum cholesterol of 20 patients with pneumonia was followed for 60 to 300 days after the onset of the illness During the febrile period there was a hypocholesterolemia as had been expected This was largely due to a marked decrease in cholesterol ester For a variable time during convalescence, wide fluctuations occurred in the serum cholesterol with, in general, a hypercholesterolemia This was due to an increase in both free and ester cholesterol, and was not associated with a fall in the basal metabolic rate or demonstrable disturbance in liver function Finally, the serum cholesterol became stabilized at a constant level assumed to be normal for the individual

The possible implication of these findings in relation to the development of atherosclerosis is discussed

A Vitamin C Saturation Test with a Modification to Compensate for the Error Due to Impairment of Renal Excretion By JOHN LUDDEN (by invitation) and IRVING S. WRIGHT New York, N. Y.

Impairment of renal function has been demonstrated to retard the excretion of vitamin C in the urine. This has resulted in erroneously low values following intravenous or oral vitamin C saturation tests. Analyses of urine specimens obtained at 1.5, 3, 5, and 24 hours after an intravenous dose of 1 gram of cevitamic acid revealed a definite correlation between (1) the percentage of the 5 hour output excreted during the first 1.5 hours, and (2) the percentage of the 24-hour output excreted during the first 5 hours.

A careful study of the data showed this correlation to hold for patients in a wide range of saturation levels and with various degrees of renal insufficiency.

A formula has been devised whereby by using the excretion figures of vitamin C for 1.5 and 5 hour samples after the test dose it is possible to predict the 24-hour output with an average error of 3.4 per cent. A patient with known renal insufficiency can be correctly evaluated as to vitamin C saturation through the use of this procedure. In our experience parallel blood and urine studies clarify the interpretation in certain instances. In certain aged individuals following the intravenous test dose it has been noted that there may be a retardation of vitamin C excretion in the absence of other laboratory evidence to suggest renal insufficiency including urea clearance and other commonly used kidney function tests.

The Urinary Excretion of Sex Hormones in Normal Children By IRA T. NATHANSON and LOIS E. TOWNE (by invitation) and JOSEPH C. AUB Boston, Mass.

Assays of the urinary estrogens, by bio-assay and of the urinary androgens by the colorimetric method were determined one or more times on 87 children under 14 years of age. There was a steady rise in the excretion of these hormones until puberty. This varied directly with the chronological age, but also with the physical maturity of the individual. There was no evidence of a cyclic excretion of the androgens in either sex. There might be daily variations in the androgen excretion but it did not appear to be of sufficient magnitude to be of physiological significance.

There was no evidence of a cyclic excretion of the estrogens in males nor in the younger females below 10 years of age. In the preadolescent years, however, there was evidence of a definite cycle of urinary estrogen excretion some time before menstruation occurred. This has been substantiated by repeated observations on the same individuals.

These normal control figures may be used as standards for the future study of the excretion of these hormones in endocrine abnormalities in childhood.

Stimulation of Skeletal Growth in Young Boys with Anterior Pituitary-like Principle By W. O. THOMPSON and (by invitation) N. J. HECKEL Chicago, Ill.

In boys showing marked genital growth during the administration of the anterior pituitary like principle from the urine of pregnant women, acceleration of skeletal growth has been observed. When the treatment was discontinued, the rate of skeletal growth decreased. In general the rate of skeletal growth appeared to bear a direct relationship to the amount of genital growth. A series of 30 treated patients varying in age from 1 to 15 years has been compared with a series of 24 untreated patients of the same age group.

Experimental Induction of Fastness to Sulfapyridine in Pneumococcus Type I By COLIN M. MACLEOD and GIUSEPPE DADDI (Introduced by O. T. Avery) New York, N. Y.

By serial transfer in serum broth containing increasing concentrations of sulfapyridine "fastness" to the drug has been induced in a strain of *Pneumococcus* Type I. "Sulfapyridine fastness" is demonstrable *in vitro* as well as in experimental infections of mice.

The sulfapyridine-fast strain of *Pneumococcus* Type I retains the morphological characteristics of the parent strain and is gram positive. No alteration in virulence or specific immunological characteristics have been demonstrated in association with the acquisition of sulfapyridine-fastness. The change in the organism appears to be a relatively permanent one.

Iodinated Protein in Human Athyroids. II. The Production of Physiological Activity by Simple Iodination of Serum Protein By J. LERMAN and W. T. SALTER Boston, Mass.

In a previous report hydrolyzed iodoprotein was shown to relieve 6 cases of human athyroidism. In terms of iodine, this material had the activity of diiodothyronine, i.e., about one-thirtieth that of whole thyroid. Recently 4 other patients have responded clinically and metabolically to oral administration of serum protein which had merely been iodinated. The original serum proved, of course, to be inert. These iodoprotein preparations contained 15 to 20 per cent iodine. It is not known definitely in what form the iodine is bound. Part of it certainly exists as diiodothyronine presumably a part exists as iodohistidine. In terms of iodine, the relative potency of this material was only one-fifth hundredth that of whole thyroid.

However, peptic digestion (as with natural thyroid) yielded a thyroxine-like fraction (active) and a diiodothyronine-like fraction (inert). The active peptone tested in three myxedematous patients and in two thyroidectomized rabbits, contained almost the entire activity of the parent iodoprotein.

This evidence that thyroidal activity can arise in serum protein through simple iodination suggests several problems: (1) Does thyronine (thyroxine minus all 4 iodine atoms) exist preformed in serum protein as an essential amino acid awaiting iodination? (2) Does the process of iodinating the protein also change molecular configura-

tion so as to produce physiological activity? (3) Can the thyroidless organism synthesize iodothyronine molecules from other iodinated residues? The proper interpretation of this chemical process may contribute to a better understanding of thyroid activity

Observations on the Inulin Clearance as a Measure of the True Glomerular Filtration Rate in Normal, Hypertensive, and Nephritic Individuals By BENJAMIN F MILLER (by invitation), ALF S ALVING, and (by invitation) M J CARL ALLINSON, Chicago, Ill

Present evidence indicates that in mammals the renal excretion of inulin equals, or closely approximates, the glomerular filtration rate. However, for man the evidence is less conclusive.

It is axiomatic that the clearance of any substance measuring only the glomerular filtration rate must be independent of the concentration of the substance in the plasma. Shannon and Smith have shown that in normal man the inulin clearance satisfies this criterion at plasma levels higher than 50 mgm per 100 cc.

The methods used in previous investigations have not allowed accurate determination of inulin at very low plasma concentrations. Also, no critical evaluation of inulin excretion as a measure of filtration in the diseased human kidney has yet been made. Employing a new method for the estimation of inulin we have determined its clearance at a range of 1 to 7 mgm per 100 cc of plasma and have compared it with the clearance at 40 to 80 mgm per 100 cc in normal subjects, hypertensive and nephritic patients. The inulin clearance values obtained at low plasma concentrations agree very closely with clearances at the higher levels, indicating that exceedingly little or no tubular reabsorption or tubular excretion of inulin occurs in the kidney of such individuals.

Observations on Effort Pain in Normal Individuals, the So-called "Stitch in the Side" with a Consideration of the Mechanism By RICHARD B CAPPS, Chicago, Ill

Although the so-called "stitch in the side" pain is extremely common, there is almost no information concerning it in the literature. Apparently, no systematic attempt has been made to observe its characteristics or to suggest a mechanism. This study is an attempt to throw some light on the problem.

Data collected by questionnaire or by asking individuals to recall their symptoms from months or years previously are notoriously unreliable in a problem of this kind. Consequently, this report is based primarily on personally observed attacks. Ninety-six such attacks have been observed in 52 different individuals. There were 43 males and 9 females. Only 2 were not healthy. Ages ranged from 15 to 63 years.

Stitch pain was produced by both mild and strenuous exertion, often only postprandial. The pain was usually located in either the right or the left upper quadrant, although a number of other loci were seen less often. Occasionally, the location varied in the same individual in different attacks. The relation to respiration was very inconstant. Bending over frequently gave relief.

Several possible mechanisms are discussed, especially the possibility of a diaphragmatic origin.

The Response of Blood Vessels in Forearm and Hand to Various Stimuli By EUGENE B FERRIS, JR, and (by invitation) DAVID I ABRAMSON, Cincinnati, Ohio

Although blood flow in the hand, as studied by plethysmography, is known to be unstable, it has under certain conditions been used as an index of peripheral blood flow. We have made a comparative study of the effects of various stimuli upon limb volume and blood flow in the hand and forearm.

Our results indicate that (1) Some stimuli cause a significant reduction in limb volume and blood flow in the hand, without notable changes in the peripheral circulation. (2) Blood flow to the forearm, under similar circumstances, is not affected although the volume often decreases. (3) Stimuli which cause a significant rise in heart rate or arterial pressure generally induce an increase in limb volume and blood flow in the forearm and a decrease in the hand. (4) The reduction in limb volume in the hand appears to be due partly to constriction of veins and partly to constriction of arterioles, whereas evidence of arteriolar constriction was never seen in the forearm. Such a dissimilarity in vasomotor reaction in the two areas is thought to be due to the presence of arteriovenous anastomoses in the skin of the palm and finger tips, and to their absence in the skin of the forearm. (5) The blood flow through the forearm is a more accurate index of peripheral blood flow than that through the hand.

Studies of Circulation and Respiration in Anxiety Neurosis and in Psychoneurosis with Anxiety Features By MANDEL E COHEN (by invitation) and JACOB E FINESINGER, Boston, Mass

Studies are in progress on circulatory function in a series of 75 patients whose diagnoses are anxiety neurosis and psychoneurosis with anxiety features. This study includes, in addition to clinical and routine laboratory observations, venous pressure, blood volume, circulation time, basal metabolic rate, minute respiratory volume, and alveolar carbon dioxide measurements. Studies of the arm to carotid circulation time (cyanide method) show a mean value of 12.6 seconds as compared with the normal value of 15.6 seconds. This indicates that the blood flows more rapidly in this group of patients than in normal individuals. Despite the rapid circulation, the oxygen consumption, as evidenced by the basal metabolic rate, is not increased (mean - 3 per cent). In a few observations of anxiety attacks, the ventilation was markedly increased (30+ liters per minute).

Changes in the Blood and Nervous System of Pigs Associated with Deficiency of Substances Contained in Yeast By M M WINTROBE and (by invitation) M SAMTER, and H LISCO, Baltimore, Md

Young pigs were weaned at 10 to 23 days of age on a diet consisting of casein, sucrose, lard, cod liver oil, a mineral mixture, ascorbic acid, and yeast. When satisfactory growth had been established the quantity of yeast given

some of the animals was gradually reduced and it was replaced by thiamin chloride, riboflavin and nicotinic acid in various combinations.

Anemia did not develop in the pigs given 3 or more grams of yeast per kgm of body weight, but it occurred in all but one of those given smaller amounts. It was characterized by the presence of macrocytes, polychromatophilia, Howell Jolly bodies and nucleated red cells and in some instances a significant increase in mean corpuscular volume was observed. In 4 of the animals in which such therapy was attempted administration of yeast was accompanied by partial or complete relief of anemia. Hyperplasia of the bone marrow was observed at autopsy in the anemic animals.

In all of the pigs given suboptimal amounts of yeast, ataxia developed and changes in the nervous system particularly in the posterior columns of the spinal cord and sensory nerves were observed. The ataxia is demonstrated in motion pictures.

The Experimental Production of Anemia in Dogs by the Injection of Sodium Iodoacetate. By J. S. WENZEL and J. L. A. FOWLER (by invitation) and J. A. DAUPHINEE and R. A. CLEGHORN Toronto Can.

In the course of some investigations to determine whether iodoacetate poisoning in dogs resembled adrenal insufficiency it was found that a marked anemia developed following subcutaneous injection of the sodium iodoacetate. No striking similarities between iodoacetate poisoning and adrenal insufficiency were observed in dogs in contrast to Verzar's observations on rats. The anemia which occurred within 10 days of the start of the bi-daily injections seems to be associated with a very marked increase in the destruction of red blood cells and increased activity on the part of the bone marrow. Death occurred as early as 10 days after the start of the injections in 2 instances, with a hemoglobin below 16 per cent. Evidence has also been obtained which indicates that this toxic action of iodoacetate is inhibited by the administration of methylene blue.

Blood and urine electrolyte studies on these animals will be made the subject of a future communication.

The Esophageal Electrocardiogram in Coronary Thrombosis. By JAN NYBOER (introduced by Herman O. Mosenthal) New York, N. Y.

Hamilton and Nyboer (1938) showed that the absence of the auricular intrinsic waves of Lewis from exploratory leads taken below the esophageal auricular border determines the position of the electrode in relation to the posterior ventricular wall in human subjects. Further study shows the validity of this method with particular reference to posterior myocardial infarct localization.

In such cases there is usually a significant Q-wave associated with R-ST segment or T wave changes in the tracings taken at the esophageal ventricular level. These were not found in normal subjects, and resemble changes found in exploratory leads over the anterior wall in cases of anterior myocardial infarction. Among these, one case is presented in which the diagnosis was established by the esophageal lead 17 years after the dramatic episode,

although healing had taken place and standard leads at this time remain equivocal.

Other factors modifying the QRS and T wave complexes in the esophageal ventricular region are also considered.

Immunological Aspects of Hemolytic Mechanism in Paroxysmal Nocturnal Hemoglobinuria. By THOMAS HALE HAM and JOHN H. DINGLE (introduced by Laurence B. Ellis) Boston Mass.

In a preliminary report (Ham T. H., New England J. Med. 1937 217 915) certain features of the mechanism of hemolysis were described for patients with chronic hemolytic anemia with paroxysmal nocturnal hemoglobinuria (Marchiafava Micheli syndrome). The fundamental abnormality resided in the red blood cells a thermolabile factor essential for hemolysis was demonstrated in plasma (heparin) and serum from five patients and from all normal subjects of compatible blood groups the patient's plasma and serum did not hemolyze normal erythrocytes. The degree of hemolysis *in vitro* and *in vivo* was influenced by variations in the acid base equilibrium.

These features suggested that an immunological reaction might be responsible for the hemolytic mechanism. No antigenic difference was observed between the abnormal and normal human erythrocytes when employed to immunize rabbits. No antibody or hemolytic substance has yet been isolated from one patient's red blood cells or stroma when treated by 10 per cent sucrose, 10 per cent salt solution dilute acid and alkali and by ether and saline. No hemolytic antibody was absorbed from the serum of one patient and of normal subjects by sheep cells or by human erythrocytes and stroma. However the serum factor essential for hemolysis was found identical in behavior to human complement since all procedures and reagents which inactivated destroyed reduced or inhibited the serum complement, or any of its four components as measured by sensitized sheep cells also reduced the hemolytic activity of the serum for the patient's erythrocytes. For hemolysis of patient's erythrocytes fresh animal serums did not restore the thermolabile components of heat inactivated human serum but fresh guinea pig serum did restore the component of human serum inactivated by ammonium hydroxide.

Vitamin A Deficiency in Diabetes Mellitus. A Photometric Study. By J. G. BRAZER (by invitation) and A. C. CURTIS, Ann Arbor Mich.

Biophotometric studies on a series of 20 juvenile diabetics were compared with similar studies on a series of 20 normals and staff members used as normals. A definite reduction in the biophotometer readings was noted in the group of diabetics.

Carotenemia was present in all the diabetics studied. It has been shown by others that the conversion of carotene to vitamin A is altered in diabetes mellitus. In substantiation of this failure to convert the provitamin carotene to vitamin A in diabetes mellitus 7 diabetics were given 60,000 units of carotene daily for 7 days, with substantial increases in their blood carotene levels but no improvement in their biophotometer readings. When

60,000 units of vitamin A were given as halibut liver oil, for a period of 7 days, the biophotometer readings showed return of function to near normal levels. Two patients receiving carotene and vitamin A supplements in similar amounts for 14 days reacted like the groups above.

Patients whose biophotometric readings were improved by the administration of vitamin A developed rapid retrogression of their biophotometric readings after the vitamin was discontinued.

Our findings suggest that juvenile diabetics are deficient in vitamin A as measured by the biophotometer in spite of high blood provitamin levels. This deficiency confirms previous work done by others that diabetics are unable to convert carotene to vitamin A.

The Histamine Content of the Blood in Allergic Disease By THERON G. RANDOLPH (introduced by Francis M. Rackemann), Boston, Mass.

Code's modification of Barsoum and Gaddum's method of determining the histamine content of blood is being used with results as follows:

1 No striking differences in the blood histamine values between normal and allergic individuals have been observed.

2 No significant increase of histamine has been found in patients suffering attacks of hay fever or asthma as compared with the same patients between attacks.

3 A few patients with very high percentages of eosinophils in their blood have been studied but failed to show any increase of histamine in the whole blood.

So far, these results are contrary to the findings of McDonald and of Code and so upset the theory that asthma depends fundamentally upon the activity of histamine. However, the work is still in progress and further data may lead to other conclusions.

Adaptation of Climate in Relation to Serum Volume By F. WILLIAM SUNDERMAN and (by invitation) H. C. BAZETT and J. C. SCOTT, Philadelphia, Pa.

Seasonal variations in the serum and blood volumes have been detected in the same individuals. These variations appear to be more marked in individuals of middle age and onward. Using an air-conditioned room it was observed that there was a gradual increase in serum volume when individuals were maintained during winter months in a hot room (33.3° C during the day and 31° during the night), and a decrease in serum volume during summer months in a cold room. At the time of increased serum volume the concentration of serum proteins was approximately normal,

the total serum protein in the circulation was therefore greater. The change in serum volume was usually correlated with a corresponding change in body weight. Infra red photographs indicate that superficial veins are not constricted fully in response to mild cold for 2 or 3 days afterwards when adjustments in blood volume have occurred. There are cardiovascular changes correlated with changes in the serum volume—thus for instance, the cardiac output was reduced or standing when the subjects were adapted to cold while the reduction was very slight when the same subjects were adapted to heat.

The Formation of Methemoglobin and Sulfhemoglobin During Sulfanilamide Therapy By J. MICHEL (introduced by W. C. L.), Durham, N. C.

Nine hundred and sixty blood samples from 6 patients receiving sulfanilamide were examined for sulfanilamide content, methemoglobin, and sulfhemoglobin. In the 277 patients who had methemoglobinemia and in the 37 patients who had sulfhemoglobinemia at some time, as demonstrable by the hand spectroscope, quantitative spectrophotometric determinations were made.

The percentage of bloods which showed methemoglobin was highest in the group that had high sulfanilamide content. The average methemoglobin value of all bloods was proportional to the sulfanilamide concentration. Methemoglobinemia did not depend upon sex, but was somewhat more frequent and more pronounced in the very young. The average methemoglobin concentration tended to diminish with increasing duration of therapy at constant blood sulfanilamide levels up to 8 mgm per cent, but at higher sulfanilamide concentrations there was a tendency for the methemoglobin to increase with time. After a single dose of sulfanilamide, the maximal methemoglobinemia occurred several hours after the blood sulfanilamide had reached its peak.

Sulfhemoglobinemia was more frequent after long courses of sulfanilamide, but did not bear any relationship to age, sex, or the concentration of sulfanilamide or methemoglobin in the blood.

On the basis of these findings, it is postulated that an active substance is normally produced in the course of sulfanilamide metabolism which causes the production of methemoglobin and sulfhemoglobin. We have demonstrated the formation of such an active substance when surviving tissues react with sulfanilamide *in vitro*. The statistics presented are found to agree with the concept that methemoglobinemia depends upon the balance of the following reactions: formation of the active agent, oxidation of hemoglobin under the influence of the active agent, and reduction of methemoglobin by the body.

THE MAGNESIUM CONTENT OF THE ERYTHROCYTES IN PERNICIOUS AND SOME OTHER ANEMIAS

By OLE BANG AND SØREN L. ØRSKOV

(From Kommunehospitalet Division III Copenhagen and Aarhus Universitet Department of Physiology Aarhus Denmark)

(Received for publication May 26 1939)

The authors have previously reported (1) variations in the permeability of the red blood cells in pernicious anemia. In spite of the considerable role played by magnesium in important fields of biology comparatively little study has been directed towards the magnesium content of the human red blood cells. Greenberg and Schmidt (2) state that magnesium is present in normal erythrocytes in quantities between 5.4 and 7.8 mgm per cent and that during anemia readings up to 15 mgm per cent may be obtained.

Animal experiments carried out by Henriques and Ørskov (3) gave results suggesting that an increased magnesium content is a property of newly formed red cells. Anemia was induced in rabbits and dogs either by bleeding or by injection of phenylhydrazine. During the regenerative stage in which the young red blood cells are relatively more numerous a considerable rise in erythrocyte magnesium was observed, occasionally after the bleeding and regularly in the phenylhydrazine experiments.

This evidence seemed to warrant the probability that information regarding human anemic states might be obtained through investigations into the magnesium content of the erythrocytes, and the following study was undertaken.

TECHNIQUE

Five to 10 cc. of blood is collected by venepuncture and defibrinated by shaking with glass beads. A hematocrit reading is performed, and the red cells are separated from plasma by centrifugalization. The magnesium content is determined by the method of Cruess and Callaghan as modified by Henriques and Ørskov (3).

RESULTS

The magnesium content of the erythrocytes was determined in 18 subjects whose blood findings were normal, 9 males and 9 females; the results are given in Table I. Values varying from 4.3 to 7.9 mgm per cent were obtained, with an average

TABLE I

Normal subjects

(This group includes patients with various diseases as well as students, and so the heading 'normal' covers the blood findings only)

Sex	Age	Hemoglobin	Erythrocytes	Magnesium
	years	per cent	millions per c. mm	mgm per cent
M	8	90	5.4	5.3
M	16	100	5.0	5.1
M	18	105	6.1	5.9
M	23	95	5.4	4.3
M	24	106	6.2	4.9
M	26	98	5.2	7.0
M	33	102	5.2	7.2
M	53	95	5.1	6.2
M	58	101	5.8	4.8
F	21	95	5.0	4.5
F	23	100	4.6	6.7
F	24	90	4.9	4.3
F	24	91	4.9	4.9
F	29	95	5.1	6.9
F	54	101	5.1	7.9
F	58	91	5.5	4.7
F	63	99	4.8	6.4
F	71	100	5.2	6.2

Minimum 4.3
Maximum 7.9
Average 5.7

of 5.7 mgm per cent. These readings correspond fairly well with those given by Greenberg and Schmidt (2) and cited above.

Ten cases of anemia from various causes were studied, the readings obtained will be seen in Table II. Among the 5 cases of anemia resulting from bleeding gastric ulcer, 2 presented values above normal *viz* Cases 1 and 2, while readings within the normal range were found in the remaining cases. Increased magnesium content was observed in a case of anemia following acute hemorrhage, Case 10, as well as in 1 case each of uremia and leukemia and in 2 cases of anemia resulting from stomach carcinoma—in one of the latter cases the highest reading 20.2 mgm. per cent, was found.

The findings in 8 cases of *pernicious anemia* are recorded in Table III. In these cases the diag-

TABLE II
Anemia from various causes

Case number	Cause of anemia	Date	Hemo- globin	Eryth- rocytes	Mag- ne- sium
			per cent	millions per c mm	mgm per cent
1	Hematemesis in peptic ulcer	June 8	59	2 7	9 3
		June 9	51	2 4	9 1
2	Hematemesis in peptic ulcer	January 14	38		10 0
		February 14	87		5 8
3	Hematemesis in peptic ulcer	April 23	41	2 4	4 1
		May 4	45	2 7	7 5
		May 10	46	2 9	4 1
4	Melena in peptic ulcer	April 21	38	2 1	6 3
		May 17	78	4 8	5 3
5	Melena in peptic ulcer	May 27	46	2 2	6 5
		May 30	50	2 9	7 4
6	Uremia	May 23	65	3 2	6 3
		June 7	58	3 0	11 9
7	Leukemia*		17	0 7	15 5
8	Carcinoma of stomach		34	2 7	20 2
9	Carcinoma of stomach		49	2 5	10 2
10	Operation with blood loss		30	2 0	14 6

* Leukocytes not thoroughly removed from the layer of red cells may constitute a cause of error

nosis was established by the hematological examination. In all but one (Case 6) there was an adequate response to liver treatment. In Case 6 the postmortem examination, including bone marrow microscopy, confirmed the diagnosis, while no other morbid state was revealed.

It will be noted that in 7 out of these 8 cases of pernicious anemia (Cases 1 to 7) the erythrocytes contained magnesium in quantities above normal, so far as the untreated stage is concerned. In 6 of the cases mentioned (Cases 1 to 6), the magnesium values decreased following liver treatment, while in 1 case (Case 7) no such change was seen.

The values obtained in Case 8 differ markedly from the findings established in the other cases. The magnesium content of the erythrocytes is exceedingly low, even below the normal limit, and no change is observed during treatment. As stated above, the course of the disease and the response to liver treatment seemed to exclude other possi-

TABLE III
Pernicious anemia

Case number	Age	Sex	Date*	Hemo- globin	Erythro- cytes	Mag- nesium
				per cent	millions per c mm	mgm per cent
1	75	M	October 7	30	0 9	14 2
			October 7--- October 18	47	1 7	5 4
2	53	M	April 12	37	1 2	15 3
			April 14--- April 15	36	1 3	12 9
			April 24	53	2 5	7 8
3	74	M	February 2	45	1 5	13 0
			February 3--- February 25	57	2 5	4 6
4	75	F	April 7	64	2 1	10 4
			April 8	62	2 0	11 2
			April 13--- April 24	72	2 6	8 6
5	70	F	January 30	63	2 6	10 1
			February 3--- February 17	68	3 3	8 1
6	68	F	March 10†	16	0 7	14 3
7	57	F	April 7	59	1 9	8 6
			April 8	59	1 9	9 4
			April 13--- April 24	71	3 0	8 5
8	80	F	November 19	36	1 4	3 8
			November 20			
			January 10	81	3 4	3 5

* ----- indicates the beginning of treatment

† Died soon after admission

bilities than pernicious anemia, and no apparent signs of complications were noted. In the erythrocytes from this case a very low content of potassium was also found, *viz*, 125 to 152 mgm per cent. A few examinations subsequently carried out indicated a gradual return to normal values. After 9 months, 64 mgm per cent of magnesium, and 396 mgm per cent of potassium were observed. Possibly a transitory "demineralization," not directly depending on the pernicious anemia, is the explanation in this instance.

COMMENT

It seems as though an increased magnesium content of the erythrocytes is occasionally but not regularly associated with anemia resulting from relatively acute blood loss. In anemias of longer

standing, an increased magnesium content may be encountered more frequently

In the anemias resulting from hemorrhage, as well as in the experimental anemias of the same origin the conception of a relative preponderance of young cells seems a justifiable interpretation in those cases where an increased magnesium content is found.

In the other anemias examined (in uremia, leukemia, and stomach carcinoma), blood loss through bleeding is not the chief cause on the contrary, a toxic inhibition of the hematopoietic function of the bone marrow is widely accepted as the dominating cause in such cases. We may assume that small portions of the marrow, still capable of producing blood cells, must counterbalance so far as possible the prevailing blood destruction. It seems, however, improbable that blood destruction should be going on at the normal rate. We feel that in the circumstances prevailing blood cells with less than the normal lifetime may occur, and so the amount of blood cells which is at any moment available for destruction may be relatively increased. Further, the toxic agents supposedly acting on the bone marrow may influence as well the circulating cells.

It will be seen that the consequence of such a *relatively increased destruction* is a condition comparable to diseases in which increased hemolysis is the primary factor, in either case the bone marrow (or what may remain of it) must increase the rate of production, and in either case the young cells predominate in the red cell population. The mechanism outlined would account for the finding of erythrocytes with a high magnesium content.

What is said above has to some extent a bearing on pernicious anemia also. Though for the moment a marrow inhibition the maturation arrest is held responsible for the development of anemia in this condition, the signs of increased blood destruction remain to be adequately accounted for. The hypothesis of accumulation of hemoglobin derived pigment (and of iron) resulting from nonutilization is not based upon sound and tenable physiological principles. Further the picture presented by the bone marrow during relapse is to the unprejudiced observer that of intensified activity, immature cells and numerous mitoses characterizing the widely expanded red marrow.

Further still when remission occurs, the signs of increased activity decrease rapidly, and in a few days the marrow regains almost normal appearance the "pernicious" character of the bone marrow is almost lost even before any rise in peripheral blood value is conspicuous. We adhere, therefore, to the formerly accepted view, and regard pernicious anemia as a condition resulting from increased blood destruction. This increased destruction, presumably, is brought about by an abnormal fragility and accordingly a short lifetime of the red cells (in the bone marrow as well as in the blood stream). The bone marrow findings during relapse are regarded as representing the utmost effort of the hematopoietic system in an endeavor to counterbalance the premature destruction of its shorthived products. As soon as active 'liver principle' is available to the marrow cells with a normal lifetime can be produced, *i.e.*, the organism is no longer 'pernicious'. This means that the hematopoietic activity may be reduced to a level corresponding to the "normal" response to the prevailing blood deficit. The remission occurs *not* as a consequence of increased activity but as the result of a stabilization of the product. The time interval between the beginning of liver treatment and the first signs of peripheral blood increase must be looked upon as representing the time needed to replace defective cells by normal ones. We have found a support for this conception in the fact, previously reported that the permeability of the erythrocytes is abnormally increased in pernicious anemia during relapse while this phenomenon disappears in the course of a few days following liver treatment. We may say that the defective 'pernicious' red cells vanish and are replaced by 'normal' cells (*i.e.* cells with a normal permeability and a normal lifetime).

The increased magnesium content of the 'pernicious' red cells found during relapse in the majority of the cases of pernicious anemia examined as well as the relative decrease in magnesium content frequently following treatment, is in keeping with the view proposed, so far as a high magnesium content is characteristic of a young blood cell.

Further investigations are needed for the evaluation of these interpretations but we feel confident that the introduction of studies

into the field of hematology will prove to be helpful in solving some of the problems outlined

SUMMARY

An increased magnesium content of the red blood cells is often, but not always, found in anemias from various causes, probably indicating a relative preponderance of young cells. In pernicious anemia, high magnesium values are found during relapse in the majority of the cases examined, while a decrease towards normal values takes place during remission. These findings support the view that a short lifetime of the red cells, with a concomitantly increased rate of destruction, is a

decisive factor in the development of the pernicious anemia

BIBLIOGRAPHY

1. Bang, O., and Ørskov, S. L., Variations in the permeability of the red blood cells in man, with particular reference to the conditions obtaining in pernicious anemia. *J. Clin. Invest.*, 1937, 16, 279.
2. Greenberg, D. M., and Schmidt, C. L. A., Occurrence, transport and regulation of calcium, magnesium and phosphorus in the animal organism. *Physiol. Rev.*, 1935, 15, 297.
3. Henriques, V., and Ørskov, S. L., Untersuchungen über den Magnesium- und den Kaliumgehalt der roten Blutkörperchen bei Anämie. *Skandinav. Arch. f. Physiol.*, 1939, 82, 86.

THE RATE OF ATTAINMENT OF DIFFUSION EQUILIBRIUM FOR THIOCYANATE BETWEEN PLASMA AND TRANSUDATES FOLLOWING THE INTRAVENOUS INJECTION OF SODIUM THIOCYANATE IN PATIENTS WITH EDEMA

By D. ROURKE GILLIGAN AND MARK D. ALTSCHULE

(From the Medical Research Laboratories of the Beth Israel Hospital and the Department of Medicine Harvard Medical School Boston)

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Estimations of the volume of extracellular fluid in man have been made by calculating the volume of body fluid through which thiocyanate is distributed after the intravenous administration of sodium thiocyanate (1, 2, 3). In the method as proposed, a known amount of sodium thiocyanate in solution is administered intravenously and blood is drawn for the measurement of the concentration of thiocyanate in the serum 1 or 2 hours after the injection (1, 2). It has been demonstrated in normal man that the distribution of thiocyanate is rapid and that diffusion equilibrium is attained within 1 hour after injection (1, 2, 3).

The time required for the attainment of equilibrium for CNS after intravenous injection of NaCNS in patients with edema has not been established, the present report affords information on this problem. Measurement of the CNS concentrations of serum and edema fluid has been made on samples drawn simultaneously at various times after intravenous injection of NaCNS.

To establish the time required for attainment of equilibrium by analyses of transudate and serum it was necessary to know what the concentration of CNS in a transudate should be in relation to the concentration in the serum, in a given system at equilibrium. Laviates, Bourdillon, and Klinghoffer (2) observed in a few experiments in edematous patients that the concentration of CNS in the serum was from 5 to 21 per cent higher than in the transudate at equilibrium when samples were drawn 12 to 64 hours after the administration of CNS. These authors suggest that CNS may combine in some manner with protein. Studies of the protein concentrations of serum and transudate have been made in each instance in the present study and the difference in CNS concentration between serum and transudate in each case has been

analyzed in respect to the difference in protein concentration.

MATERIAL AND METHODS

Twenty-three studies have been made in 13 patients with edema caused by congestive heart failure, cirrhosis of the liver carcinoma, hepatic vein thrombosis, or vena caval obstruction. In 8 studies edema fluid was obtained from the abdomen, in 7 from the thoracic cavity, in 8 from the subcutaneous spaces. Subcutaneous edema fluid was removed by means of Southey's tubes.

From 15 to 20 cc. of 5 per cent sterile solution of NaCNS was injected intravenously in approximately 3 minutes, the amount depending on the body weight of the patient and the estimated volume of edema fluid. Samples of blood and edema fluid were obtained simultaneously from 1 to 28 hours after the intravenous injection of NaCNS, in several instances 2 or more samples of blood and fluid were drawn at various times after an injection. The concentrations of CNS in the sera and transudates and in urine were measured as described by Laviates, Bourdillon, and Klinghoffer (2).

Before the data could be applied to the study of the rate of attainment of equilibrium it was necessary to determine the nature of the distribution of CNS when equilibrium is reached. The distribution of CNS in relation to the distribution of protein between the sera and fluids was investigated from this point of view. The total protein concentrations of sera and transudates were estimated from specific gravity measurements made according to Moore and Van Slyke (4) using 5 cc. specific gravity bottles. The formula,

Grams of total protein per 100 cc.

$$= \frac{34.3}{G - 1} \times W$$

where G represents specific gravity, was employed (4)¹

The "available fluid" for CNS distribution (1) was calculated by the formula,

$$\text{Liters of "available fluid" = } \frac{\text{mgm of CNS in body}}{\text{mgm of CNS per liter of serum}}$$

¹ This formula was applied by Moore and Van Slyke (4) to plasma analyses. Analysis of data obtained earlier in this laboratory (5) demonstrates that the formula is applicable to approximate estimations of the protein concentrations of edema fluids. The values were available for protein concentrations as measured by the macro-Kjeldahl method, and for specific gravity as measured according to Moore and Van Slyke, in 22 thoracic or abdominal fluid specimens. In this series the average protein concentration was 203 grams per 100 cc., the limits being 0.6 to 4.4 grams per 100 cc. As calculated from the specific gravity values, employing the formula above, the average estimated protein concentration was

From this value and the surface area of the patient the liters of "available fluid" per square meter of body surface were calculated. The amount of CNS in the body was calculated from the amount injected minus the amount which had been excreted in the urine up to the time of withdrawal of the sample of blood. The amounts excreted in the urine were variable, but small, for the periods of study of 11 hours or less, the greatest excretion rate was 30 mgm in 6 hours.

RESULTS

After the intravenous injection of NaCNS the concentration of CNS increased slowly in the transudates of patients of this study, as shown by serial samplings of transudate and blood in 4 cases (Figure 1)

200 grams per 100 cc. The standard deviation of the differences between the 2 methods was 0.25

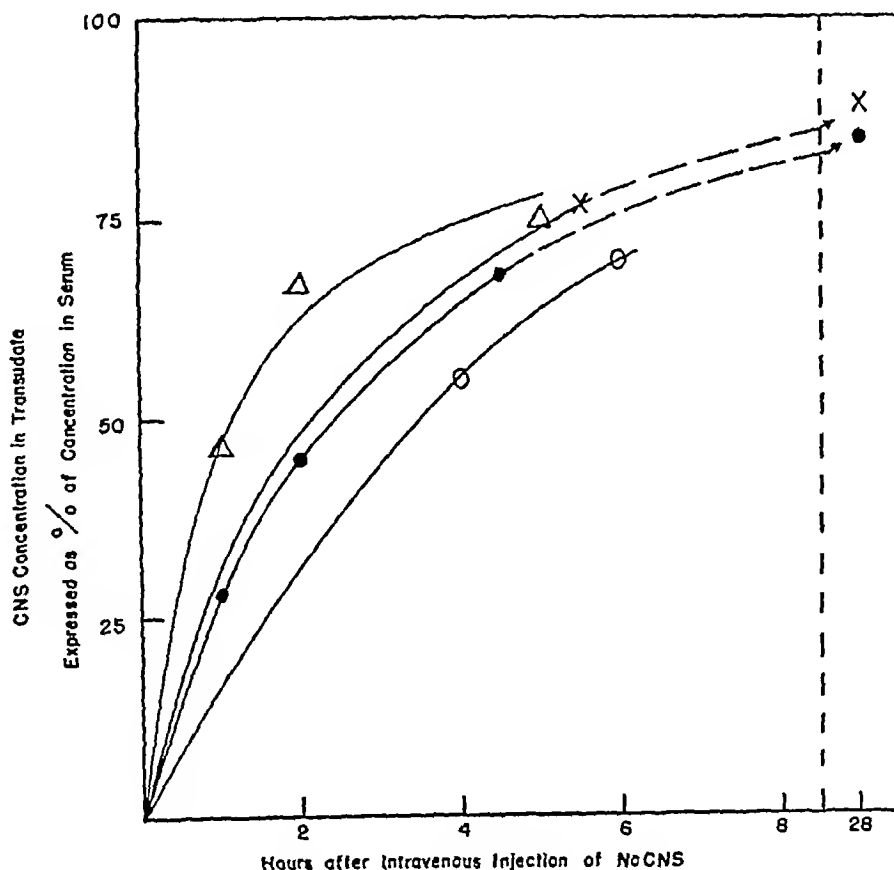


FIG 1 RATE OF APPROACH TO DIFFUSION EQUILIBRIUM FOR CNS AS SHOWN BY ANALYSES OF SERIAL SERUM AND EDEMA FLUID SAMPLES DRAWN IN 4 PATIENTS AT VARIOUS TIMES FOLLOWING INTRAVENOUS INJECTION OF NaCNS

In 5 cases in which samples of transudate and blood were drawn from 8.5 to 28 hours after the intravenous injection of NaCNS the CNS concentrations in the sera ranged from 1 to 20 per cent higher than the concentrations in the corresponding transudates (Figure 2). In 2 of these experiments subcutaneous edema fluid was studied, in 2 chest fluid, and in 1, ascitic fluid. The protein concentrations in the sera in these cases varied from 1.3 to 5.5 grams per 100 cc. higher than in the corresponding transudates. When the concentration of CNS in the transudates expressed as the per cent of the concentration in the corresponding sera, is plotted against the excess concentration of protein in the sera (grams of serum protein per 100 cc. minus grams of transudate protein per 100 cc.) a straight line relationship is observed in these 5 cases (Figure 2—letters C).

In 2 of 3 cases studied 6 hours after the injection of CNS the concentrations of CNS in the sera were 5 and 12 per cent higher than in the transudates. The same relationship to the

protein values existed in these cases as in those above described (Figure 2), it may be inferred then that equilibrium was attained also in these 2 instances. In the other case studied after 6 hours the relative concentration of CNS in the transudate was considerably lower than that expected at equilibrium (Figure 1 Case A). Of the 9 studies made from 3.5 to 5.5 hours after injection equilibrium had been attained in only 3 (Figure 2). Equilibrium was not attained in any of the 6 experiments in which 1 to 3 hours elapsed between injection and sampling. A summary of all these findings is given in Figure 3.

In several of the studies made on samples drawn 5.5 hours or less after the injection of NaCNS, the CNS concentrations in the transudate were so low as to indicate that equilibrium would not have been attained until 8 to 10 hours after the injection. For example, in 2 patients with ascites from whose abdomens 6 and 7 liters of fluid were drawn the concentrations of CNS in the ascitic fluids 4 hours after administering NaCNS were only 50 and 55 per cent of the

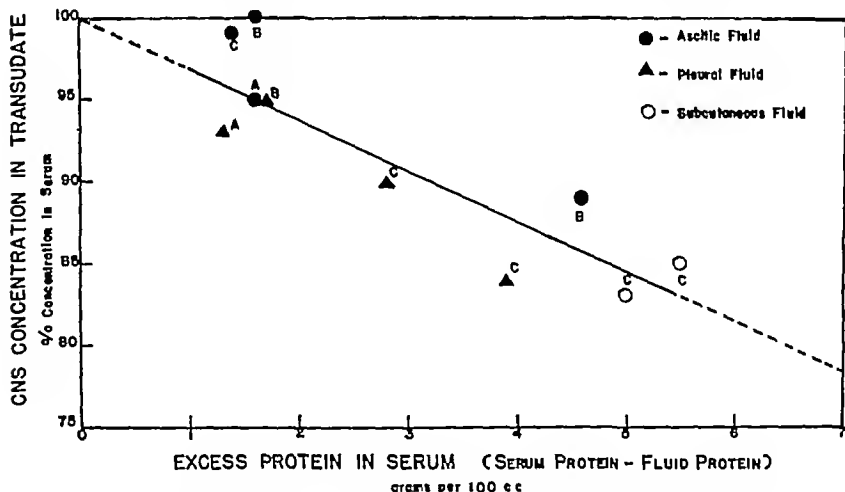


FIG. 2. RELATIONSHIP BETWEEN THE DIFFERENCE IN PROTEIN CONCENTRATION IN SERUM AND TRANSUDATE AND THE DIFFERENCE IN THE CNS CONCENTRATION OF SERUM AND TRANSUDATE

The letters refer to the time elapsed between intravenous injection of NaCNS and collection of samples. A = 4.0 to 4.5 hours, B = 5.5 to 6.0 hours, C = 8.5 to 28 hours.

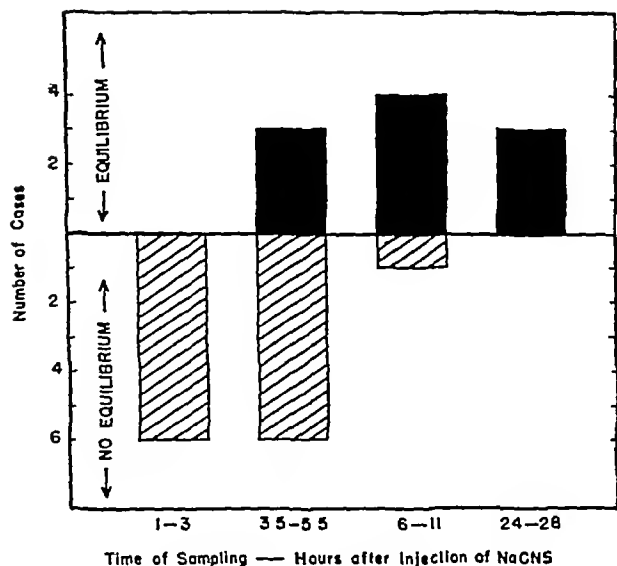


FIG 3 TIME REQUIRED FOR ATTAINMENT OF DIFFUSION EQUILIBRIUM FOR CNS IN VARIOUS EDEMATOUS DEPOSITS, FOLLOWING THE INTRAVENOUS INJECTION OF NaCNS IN PATIENTS WITH EDEMA

concentrations in the sera. Distribution was complete in a shorter time in patients with smaller amounts of ascites.

Approximate equality of the concentrations of CNS in two blood samples drawn 2 hours and 4 hours after the injection of NaCNS was observed in 2 instances in which the edema fluid sample drawn at 4 hours showed a CNS concentration lower than that to be expected at equilibrium.

The "available fluid" for CNS distribution in the experiments in which equilibrium had been reached varied from 29 to 59 per cent of the body weights of the patients, or from 11 to 24 liters per square meter of body surface. These values are to be contrasted with the reported normal values of 24 per cent of body weight, or 9.5 liters per square meter of body surface (1). The smallest increases above the normal in "available fluid" were obtained in patients with carcinomatous lymphatic obstruction limited to one pleural space, the greatest increases were observed in patients with congestive heart failure and generalized anasarca.

DISCUSSION

The above data demonstrate that diffusion equilibrium for CNS usually is not attained in ede-

matous patients with large volumes of fluid in the thoracic, abdominal, or subcutaneous spaces until 6 to 10 hours following the intravenous injection of CNS. The shortest time observed for the establishment of equilibrium was 4 hours, in this instance fluid in the thoracic cavity of a patient with carcinomatous obstruction was studied.

The time required for attainment of diffusion equilibrium for CNS in a given body cavity depended upon the volume of edema in that space. It is true, probably, that equilibrium is reached more quickly in a patient with a given amount of edema spread over the several body compartments available for extracellular fluid deposits, than in a patient with a similar or even somewhat lesser total amount of edema which is contained mainly in the abdominal or thoracic cavity. Obviously equilibrium would be attained more rapidly than observed here in edematous patients with lesser amounts of fluid than those of this study, and more slowly in patients with greater edematous deposits.

Because of the above considerations it is suggested that in routine studies of the amount of "fluid available" for the distribution of CNS in edematous patients, blood for serum CNS measurement should not be drawn until 12 to 24 hours after the intravenous administration of the salt, this period is probably also of sufficient duration when the salt is administered orally. Under these conditions the amount of CNS excreted in the urine should be measured. The approximate equality of concentration of CNS in serial samples of serum drawn at 2-hour intervals following injection, does not necessarily imply that equilibrium has been attained throughout the body in patients with edema. The concentration in a transudate increases slowly during the later period of equilibration (Figure 1), the CNS thus entering a given edematous deposit may be obtained not only from the blood but also from the more quickly equilibrated extracellular spaces in less edematous parts of the body. The decrease in CNS concentration of the blood under these circumstances, therefore, would be relatively small over a period as short as 1 to 2 hours.

In the cases of this study in which equilibrium was attained, the concentrations of CNS of the sera ranged from 0 to 20 per cent higher than

those of the corresponding transudates. The data of Figure 2 suggest that the excess CNS in the serum, as compared with that in the transudate, may be related to the higher concentration of protein in the serum,² that this relationship might exist was suggested previously by Laviates Bourdillon, and Klinghoffer (2). Conceivably, CNS may be bound by some other component of the serum which is distributed between plasma and transudate in a manner like protein. It has been suggested (2) that the lower concentration of CNS in extracellular fluid be considered in calculations of extracellular fluid volume. However, since the ratio of concentration of CNS in extracellular fluid to that in serum in normal man cannot be established directly and further, since this ratio is variable in patients with edema, and, undoubtedly even in different compartments in a given patient with edema the authors do not consider such a correction valid. Thiocyanate enters the red blood cells but does not enter other cells in the body (1, 2, 3). It has been suggested that a correction be made in calculating extracellular fluid volume for the CNS contained in the red blood cells (2), this correction could not be applied in patients with edema and abnormal blood volumes unless the blood volumes were accurately measured. For these reasons we have calculated simply the available fluid for distribution of CNS as originally suggested by Crandall and Anderson (1) (see methods).

It has been noted in normal man that CNS is distributed in the body somewhat more rapidly than sucrose or sulfate (2). Likewise, the comparative rate of distribution of various substances in patients with edema undoubtedly varies with the coefficient of diffusion of the substance. It has been pointed out that because of the relatively slow diffusion rate and the rapidity of excretion of sulfate and sucrose from the body, these substances cannot be used to measure extracellular fluid volume in patients with edema (2).

In all studies of the distribution of substances at equilibrium between plasma and transudates in

edematous patients the relatively long time required for the attainment of diffusion equilibrium must receive consideration. It was observed earlier by one of us (5) that the concentration of glucose in the water of transudates of edematous patients was sometimes higher than that in the water of the serum after a fast of 12 or some few more hours. The greatest differences were observed in edematous diabetic patients. This finding was attributed to the absence of diffusion equilibrium for glucose, referable to the time required for equilibration, and the relatively great variations in blood sugar which occur rapidly particularly in diabetic patients (5).

SUMMARY AND CONCLUSIONS

1 In the patients of this study with large accumulations of edema fluid due to various diseases diffusion equilibrium for CNS between plasma and transudates in the subcutaneous, thoracic, or abdominal cavities usually was not attained until 6 to 10 hours after the intravenous injection of NaCNS. This finding is contrasted to the observation that diffusion equilibrium for CNS obtains in normal subjects within one hour after the injection of NaCNS.

2 The time required for attainment of diffusion equilibrium for CNS between plasma and the edema fluid in a given body compartment appears to be directly proportional to the volume of transudate in that compartment.

3 At equilibrium the concentrations of CNS in the sera were from 0 to 20 per cent higher than the concentrations in the transudate. Studies of the relative concentrations of protein in sera and transudates indicate that some CNS is "bound" by protein or some other relatively non-diffusible substance.

4 It is suggested that in routine studies of the amount of "available fluid" for CNS distribution in edematous patients, 12 to 24 hours should elapse between the administration of the CNS and the drawing of blood for measurement of the CNS concentration in the serum.

5 In all studies of the distribution of substances at equilibrium between plasma and transudates in edematous patients, the relatively long time required for the attainment of diffusion equilibrium must receive consideration.

² A closer correlation than appears in Figure 1 might be evidenced if the concentrations of protein were measured directly by the Kjeldahl method, instead of calculated from measurements of specific gravity. Further the relationship may be affected by the proportions of excess albumin or globulin.

PLAN OF STUDY

Between May 1937 and May 1938, an attempt was made to obtain frequent blood specimens of all patients receiving sulfanilamide in the clinics and on the wards of Duke Hospital. The drug was generally given by mouth in equal doses four to six times daily. The blood specimens were drawn, when possible, between three and five hours after the ingestion of the drug and were examined shortly thereafter.

The free whole blood sulfanilamide concentration was determined by the method of Marshall (16). At least two standards were used because of the slight deviations from Beer's law. The bloods were examined qualitatively for the presence of abnormal pigments (17). The absorption spectra were inspected through a hand spectroscope for abnormal bands in the red region. The alpha methemoglobin band was confirmed by the usual tests (addition of cyanide in all cases, occasionally supplemented by the action of alkalis and reducing agents). The alpha sulfhemoglobin band was checked by its stability to these reagents.

bands were recognized if more than 4 to 6 per cent of the total pigment had been converted to methemoglobin or if more than 1 per cent was present as sulfhemoglobin. If the abnormal bands were detected, the bloods were subjected to quantitative spectrophotometric examination (17). The method for methemoglobin depended either on the ratio of the extinctions of oxyhemoglobin and cyanmethemoglobin at wavelengths of 575 $m\mu$ and 560 $m\mu$ or on the specific change in absorption at a wavelength of 634 $m\mu$ upon the conversion of methemoglobin to cyanmethemoglobin. The method for sulfhemoglobin depended upon the ratio of the extinctions of sulfhemoglobin and oxyhemoglobin at wavelengths of 540 and 560 $m\mu$. All quantitative determinations were performed on the Bausch and Lomb Universal Spectrophotometer.

Normal bloods never showed any change in absorption at 634 $m\mu$ upon the addition of cyanide, nor did the blue product formed from sulfanilamide by the action of ultraviolet light. Since all quantitative determinations were preceded by confirmatory qualitative tests, it is almost certain that the methods were specific for the pigments in question.

RESULTS

A Methemoglobin formation

There were 960 blood examinations made on 476 patients. Methemoglobin, in quantities demonstrable by the hand spectroscope, was found in the red cells of 277 or 58 per cent of the patients at some time during the administration of sulfanilamide. The distribution of these patients according to age and sex is shown in Figure 1. The per cent of patients developing methemoglobinemia is roughly equal in the sexes, but is higher in the very young.

Effect of blood sulfanilamide concentration on methemoglobinemia

All blood specimens were divided into five groups on the basis of their sulfanilamide concentration. In each group, the percentage of bloods which showed methemoglobin (in sufficient quantity to be detected by the hand spectroscope) was calculated. These are plotted against the sulfanilamide concentration of the group in the upper curve in Figure 2. In the 0 to 4 mgm per cent sulfanilamide group, 39 per cent of the specimens were positive. This percentage increased with increasing sulfanilamide concentration until 100 per cent was reached in the group of bloods showing over 16 mgm per cent sulfanilamide.

In each sulfanilamide concentration group, the average methemoglobin value of all bloods within that group was calculated. It can be seen in Figure 2 (lower curve) that the average methemoglobin value varies directly as the sulfanilamide concentration of the blood. It is to be clearly understood that, throughout this study, only those bloods were considered positive for methemoglobin which showed the pigment in amounts detectable by the hand spectroscope. Since quantitative examination was limited to these bloods, the average methemoglobin values herein reported are not true averages. Specimens, calculated as not containing methemoglobin, may have contained up to approximately 0.6 gram per cent. Undoubtedly, the number of positive bloods and the average methemoglobin levels would have been raised if specimens with smaller amounts of methemoglobin had been subjected to quantitative determination. Nevertheless, it is obvious that there is very direct correlation between the average methemo-

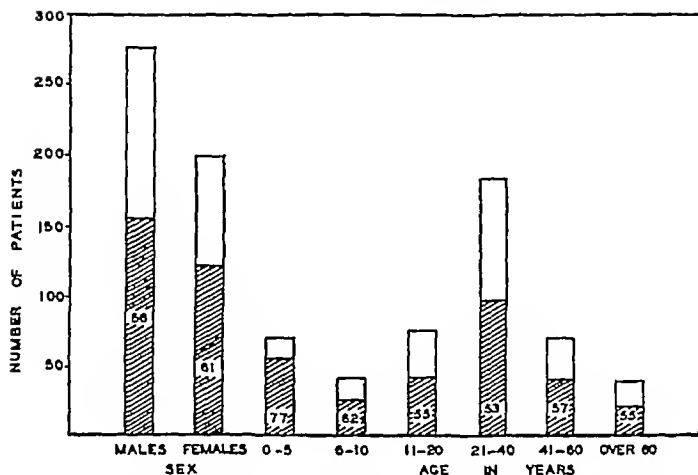


FIG. 1 THE INCIDENCE OF METHEMOGLOBINEMIA, ACCORDING TO SEX AND AGE

Total height of column gives the number of patients studied in each group. The shaded portion gives the number of patients showing methemoglobinemia at some time during therapy. The numbers in the shaded areas refer to the percentage of patients in each group that showed methemoglobinemia.

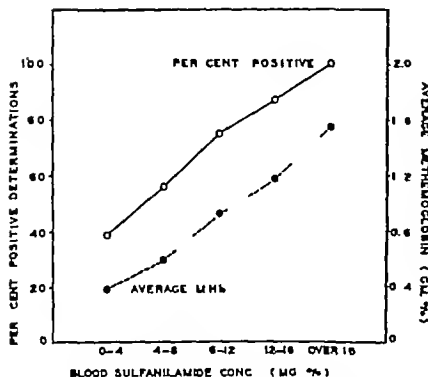


FIG. 2. THE EFFECT OF BLOOD SULFANILAMIDE CONCENTRATION ON METHEMOGLOBINEMIA

Upper curve The percentage of blood specimens showing methemoglobin. Lower curve The average methemoglobin concentration of all blood specimens in each blood sulfanilamide concentration group

globin level and the concentration of sulfanilamide in the blood. At sulfanilamide concentrations in excess of 16 mgm per cent methemoglobin was invariably present in easily demonstrable quantities. The maximum methemoglobinemia was 5 grams per cent—a conversion of over 40 per cent of the total hemoglobin. This occurred at a blood sulfanilamide concentration of 18 mgm per cent.

Effect of age and sex upon the development of methemoglobin

It has already been shown that the percentage of patients developing methemoglobinemia is roughly equal in the sexes but is higher in the very young. These conclusions have to be reexamined with regard to the blood sulfanilamide concentrations since it is possible that the drug may have been given in larger amounts and may have reached higher blood levels in a particular group. All the blood specimens in each age and sex group were therefore subdivided according to their sulfanilamide concentration. Table I shows the per

dures Apparently the blood hemoglobin levels were rather evenly distributed through the age, sex, and sulfanilamide concentration groups

The effect of disulfanilamide and sulfapyridine on the development of methemoglobin

Seventeen patients received courses of disulfanilamide. Fourteen or 83 per cent developed methemoglobinemia during the medication. Of the remaining three, two had received previous courses of sulfanilamide without showing methemoglobin. Of the fourteen showing methemoglobinemia, four had previous courses of sulfanilamide without showing methemoglobinemia. The methemoglobin concentrations reached in the equivalent sulfanilamide and disulfanilamide level groups were distinctly higher in the case of disulfanilamide. It is therefore suggested that the tendency to methemoglobin formation is greater with disulfanilamide than with sulfanilamide.

Preliminary studies on the action of sulfapyridine show that the tendency to cyanosis and to the development of methemoglobin is distinctly less with this substance than with sulfanilamide, though both do occur.

Rapidity of development of methemoglobinemia

Several patients were given a single oral dose of 3 grams of sulfanilamide or disulfanilamide. Blood samples were taken at hourly intervals for 5 to 7 hours. In contradistinction to the procedure generally followed in this study, all bloods were examined quantitatively for abnormal pigments whether or not the pigment was demonstrable by the hand spectroscope.

Case 1

Age 31 years Weight 55 kilos Diagnosis Testicular adenocarcinoma Dose 3 grams sulfanilamide Total hemoglobin 13.1 grams per cent

Hours after taking drug	Free blood sulfanilamide mgm per cent	Methemoglobin grams per cent
1	1.9	0.42
2	4.9	0.95
3	5.4	1.34
4	4.6	1.41
5	4.4	1.68
27	1.0	0.62

One week later, a similar test was made with 3 grams of disulfanilamide.

Hours after taking drug	Blood dl sulfanilamide mgm per cent	Methemoglobin grams per cent
1	1.6	0.17
2	2.8	0.93
3	3.6	1.91
4	3.8	2.73
5	3.6	4.84
6	3.0	3.43
7	3.0	3.14

Case 2

Age 39 years Diagnosis Carcinoma of prostate, urinary infection Dose 3 grams sulfanilamide Total hemoglobin 16 grams per cent

Hours after taking drug	Blood sulfanilamide mgm per cent	Methemoglobin grams per cent
1	3.7	0.24
2	4.9	0.34
3	5.3	0.34
4	4.2	0.43
5	3.7	
6	3.5	0.46
7	3.4	0.37

Case 3

Age 74 years Diagnosis Carcinoma of prostate, urinary infection Dose 3 grams disulfanilamide Total hemoglobin 9.4 grams per cent

Hours after taking drug	Blood disulfanilamide mgm per cent	Methemoglobin grams per cent
1	0.6	0
2	1.0	0
3	2.2	0
4	2.2	0
5	2.6	0
6	2.6	0.28
7	2.6	0.37
24	1.8	0.56

In these patients, methemoglobin formation is rapid but does not reach its height until at least 1 to 2 hours after the blood sulfanilamide is maximal. This delay is more prominent with disulfanilamide than with sulfanilamide. In the first case where the disulfanilamide and sulfanilamide were given under comparable conditions, methemoglobin formation was very much more pronounced with disulfanilamide. This is in accord with the conclusions previously deduced. The rate of disappearance of the methemoglobin must be very rapid since, in the first case, the methemoglobin percentage dropped from 1.68 grams per cent to 0.62 gram per cent in the course of 24 hours even though sulfanilamide was still present in the blood. This agrees with our experience that methemoglobin is almost invariably absent within 24 to 72 hours after the cessation of medication.

Discussion on methemoglobinemia

Résumé of literature

The conversion of hemoglobin to methemoglobin involves the oxidation of the ferrous iron to the ferric state (18). A number of oxidizing substances can rapidly oxidize hemoglobin: ozone, iodine, chlorates, ferri cyanides, hydrogen peroxide, permanganates, hydroquinone, hydroxylamine, p-aminophenol and others (19). In the presence of oxygen, hemoglobin is gradually converted to methemoglobin. In whole unhemolyzed blood this conversion is very slow and must, in part, be delayed by the presence of anti-oxidants or reducing substances. Glutathione, glucose, or unsaturated fatty acids may play such a rôle in the whole blood (20). In the intact or ganism, methemoglobin is very rapidly reduced to hemoglobin and it is probable that other reducing systems are of importance.

Clinically a number of substances have been noted to produce methemoglobinemia in humans and animals. To one group belong obvious oxidizing agents such as potassium perchlorate. The action of these substances is easily explicable since they are sufficiently strong oxidizing agents to perform the conversion *in vitro* as well as *in vivo*. Another group of compounds has been reported to cause cyanosis and methemoglobinemia *in vivo*—e.g. aniline, acetanilid, nitrobenzene (21). These substances are not in themselves oxidizing agents and thus cannot form methemoglobin *in vitro* from hemoglobin. It has been therefore postulated that these substances are metabolized by the body to some active oxidizing agent which is sufficiently powerful to produce the methemoglobinemia. Ellinger (26) believed that phenylhydroxylamine was the active agent since he found acetophenylhydroxylamine in the blood of animals poisoned with acetanilid. Lipschitz (27) believed that a similar substance was the active agent in nitrobenzene poisoning and he was able to produce nitrophenylhydroxylamine on shaking dinitrobenzol with frog muscle *in vitro*. Heubner and his co-workers (28) however disagreed with these conclusions and believed that p-aminophenol was the active substance. The presence of this substance has been demonstrated in the blood and urine of animals after aniline or acetanilid ingestion by Young (22a), Herrick and Irons (29) and by Lundstein, Meulengracht, and Rischel (23). Heubner and his co-workers (28) also pointed out that nitrophenyl hydroxylamine could act only in molar proportions with hemoglobin since the final product was inactive azo-oxybenzol. On the other hand, p-aminophenol forms a reversible redox equilibrium with quinone so that one mole of p-aminophenol could produce many moles of methemoglobin. This was found to be true in aniline poisoning by Schwedtke (30). The probable appearance of aminophenols has been demonstrated *in vitro* through the action of frog muscle on dinitrobenzol by Heubner and Lo-Sing (31) and through the action of rat liver on acetanilid by Michel and Bernheim (32).

It is interesting that the same confusion has arisen in regard to the cause of cyanosis after aniline ingestion as in the question of sulfanilamide cyanosis. Young and

his co-workers (22) came to the conclusion that neither acute nor chronic poisoning with acetanilid or aniline is accompanied by the formation of methemoglobin. They believed that phenolic oxidation products stained the red cells and were able to show accumulation of phenolic substances in the blood after aniline poisoning. Lundstein, Meulengracht, and Rischel (23) came to the conclusion that oxidation products of aminophenol stained the red cells in their case of human acetanilid poisoning although they found 18.5 per cent of the hemoglobin had been converted to methemoglobin (by Van Slyke and Hiller method) (24). They found that serum and urine gave positive tests for p-aminophenol and argued that this substance had diffused into the red cells and had become oxidized to dark material. On the other hand, there have been very many reports of methemoglobinemia following ingestion of one of these compounds (21). Part of the discrepancies may be explained by differences in the animals used since Levin (25) has shown that cats, dogs and rats develop methemoglobinemia on administration of nitrobenzene whereas the rabbit and guinea pig do not. The duration of some of the animal experiments may have been too short to allow the production of methemoglobin.

If an active agent is formed from any of these substances, the extent of methemoglobin formation will depend primarily upon (a) the speed of formation of the active agent, (b) the speed of interaction between the active agent and hemoglobin, (c) the rate of destruction of the active agent, and (d) the rate of reduction of methemoglobin. Variations in these factors may explain the differences in methemoglobinemia found in patients after the ingestion of acetanilid or aniline. For example, Schwedtke (33) showed that alcohol or ascorbic acid intensifies the toxic action of aniline probably because they prevent the oxidative destruction of the active agent.

It has been demonstrated that 58 per cent of an unselected group of patients receiving sulfanilamide have shown methemoglobinemia, the intensity of which is, on the average, proportional to the concentration of sulfanilamide in the blood. It is probable that many more patients would show methemoglobinemia if sufficient examinations had been made and if our methods detected very small quantities of methemoglobin. Methemoglobin formation in the course of sulfanilamide administration is therefore to be considered as a definite action of the drug and not as an idiosyncrasy on the part of certain patients. Since sulfanilamide cannot oxidize hemoglobin *in vitro* (unpublished experiments) we were led to postulate that some active agent was formed during the course of metabolism of sulfanilamide by the body which could directly or indirectly cause the oxidation of hemoglobin to methemoglobin. Such mechanism

has already been suggested to account for methemoglobin formation after acetanilid or aniline ingestion

This hypothesis was found to agree very well with the observed data

1 The extent of methemoglobinemia depends upon the blood sulfanilamide concentration since the speed of the reaction concerned in forming the active agent will naturally depend upon the concentration of substrate. Since sulfanilamide is approximately equally distributed in all body tissues and is stored by none (34), the concentration of sulfanilamide in the active tissue will be reflected by the concentration in the blood

2 The lag period between the maximum sulfanilamide concentration and the maximal methemoglobinemia after a single dose of sulfanilamide may be, in part, due to the time necessary for the formation and accumulation of sufficient quantities of the active agent

3 We have observed that the amount of methemoglobin tends to increase as the hemoglobin content increases—so that the average ratio of methemoglobin to hemoglobin remains relatively constant in a series of cases at a definite sulfanilamide level. The speed of the reaction between the active agent and hemoglobin might be increased by greater concentrations of hemoglobin and thus could cause this effect

4 The absence of a cumulative effect in the production of methemoglobin may be explained by the lability of the active agent (in the event that the agent functions as a catalytic system) and by the ability of the body to reduce methemoglobin back to hemoglobin in a relatively short time. At very high blood sulfanilamide levels, a tendency for a cumulative effect should arise when the speed of formation of the active agent exceeds the body's defenses. This has been noted in certain cases where the dose of sulfanilamide has had to be reduced because of the progressively severe methemoglobinemia. Usually, however, the defenses of the body (destruction of the active agent and reduction of methemoglobin) are mobilized more actively during a course of sulfanilamide since the average methemoglobin tends to diminish with time even though the blood sulfanilamide level remains constant

5 The variations in the ability of persons to form methemoglobin at definite blood sulfanil-

amide concentrations will naturally depend upon the usual biological variations in the several processes concerned. These processes may be influenced by inherent ability, tissue damage (in regard to the reduction of methemoglobin), or by the state of nutrition. (Schwedtke's experiments on the effect of ascorbic acid have already been cited.) It is conceivable that young infants show more methemoglobin than the older groups because of their more active metabolism and the resultant increased quantities of active agent formed

B Sulfhemoglobin formation

Thirty-seven or 7.8 per cent of the 476 patients in the series developed sulfhemoglobinemia at some time during their course of therapy

Influence of drugs

Of the thirty-seven patients showing sulfhemoglobinemia, one had received sulfarsphenamine and guaiacol, two had received 0.9 gram of ferrous sulfate daily and two had received 15 grams of magnesium sulfate on the first day of sulfanilamide therapy. One of the latter did not develop the sulfhemoglobinemia until 3 to 6 days after the administration of the magnesium sulfate. This makes it unlikely that the salt was an important factor in the abnormal pigment production in that case. The ferrous sulfate can be eliminated as an important factor since 16 patients of the entire group of 476 patients received ferrous sulfate and only 2 developed sulfhemoglobinemia while 7 showed methemoglobin formation. Neither incidence differs appreciably from the general average for the remainder of the patients. As would be expected, acetanilid seems to predispose to sulfhemoglobin formation since two patients, who had been taking acetanilid and bromides just prior to the sulfanilamide therapy, developed sulfhemoglobinemia. Drugs which did not predispose to abnormal pigment production are neoarsphenamine, bismuth, barbiturates, and morphine derivatives. Specific serum and heat therapy were given to several patients without untoward effects. Constipation was not more prominent in the groups showing abnormal blood pigments

Influence of blood sulfanilamide concentration

Attempts to correlate the sulfhemoglobinemia with the blood sulfanilamide concentrations were

unsuccessful Of the 88 examinations which were performed on the 37 patients developing sulfhemoglobinemia, 73 per cent were positive for sulfhemoglobin. This percentage was not significantly changed when the bloods were divided into groups based on the blood sulfanilamide concentrations A scatter diagram of the blood sulfhemoglobin levels plotted against the blood sulfanilamide concentration confirms our impression of the lack of correlation between these two values (Figure 4)

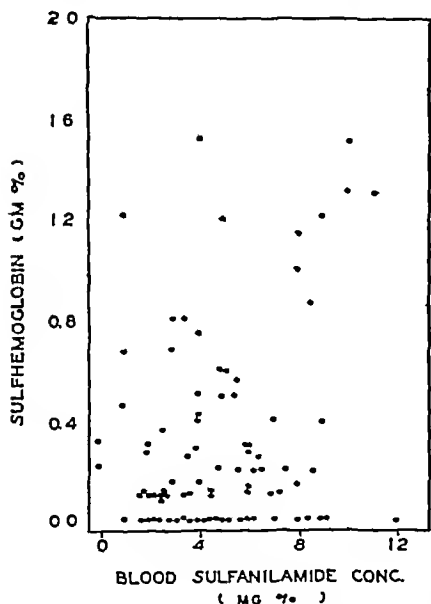


FIG. 4 THE EFFECT OF BLOOD SULFANILAMIDE CONCENTRATION UPON SULFHEMOGLOBINEMIA

Spot diagram of all determinations on patients who showed sulfhemoglobin at some time during sulfanilamide therapy

Effect of duration of therapy

Several blood examinations were made upon 21 of the 37 patients who developed sulfhemoglobinemia. Nine of these patients showed sulfhemoglobin at all examinations while 9 developed sulfhemoglobinemia only during the latter days of

therapy (usually after the first week) Three patients developed minimal amounts of sulfhemoglobin during the first week which disappeared while the patients were still taking sulfanilamide. It thus seems that patients are somewhat more likely to show sulfhemoglobin after longer courses of the drug The question could not be investigated more thoroughly because of the paucity of data.

After the drug is discontinued, the sulfhemoglobin tends to persist for a rather long time. In one case, for example the sulfhemoglobin concentration dropped slowly from 0.7 to 0.2 gram per cent in the course of 6 days after stopping the sulfanilamide therapy

Influence of sex and age

There are no significant differences among the incidences of sulfhemoglobinemia among the sexes Eight per cent of the males and 7.5 per cent of the females developed sulfhemoglobin at some time during the course of therapy Likewise it could not be demonstrated that age had any pronounced effect.

Effect of hemoglobin concentration

A scatter diagram failed to show any correlation between the hemoglobin concentration and the degree of sulfhemoglobinemia in the blood specimens that were obtained on the patients who showed sulfhemoglobin at some time during the course of therapy

Relationship of sulfhemoglobin and methemoglobin formation

A scatter diagram did not demonstrate any parallelism in the development of sulfhemoglobin and methemoglobin Patients with intense sulfhemoglobinemia often did not show methemoglobin. The maximum sulfhemoglobin concentration in this series was 1.52 grams per cent (a conversion of 14 per cent of the total pigment) This was associated with 1.8 grams per cent methemoglobin on one occasion and with a negative test for methemoglobin at another time in the same patient The independence of sulfhemoglobin and methemoglobin formation is also seen in the fact that the direct dependence of methemoglobin on sulfanilamide concentration was present in the sulfhemoglobin group of patients even though the

sulfhemoglobin concentrations did not show this dependence

Discussion on sulfhemoglobin formation

Résumé of the literature on sulfhemoglobin

The constitution of sulfhemoglobin is not known. Apparently its formation involves the introduction of one atom of labile sulfur and some change in the linkage of the globin to the heme portion of the molecule. This change is irreversible and there is no method for converting sulfhemoglobin back to hemoglobin either *in vivo* or *in vitro* without disrupting the molecule (35)

The first definite clinical case of sulfhemoglobinemia was reported by van den Bergh in 1905 (36). At that time, although it was recognized that sulfides in the intestines might be absorbed and cause sulfhemoglobinemia, the explanation for its formation was not clear since it had been demonstrated that the inhalation of hydrogen sulfide in warm blooded animals did not lead to the formation of sulfhemoglobin if the animals survived (37). In 1907, Clarke and Hurlley (38) studied the *in vitro* formation of sulfhemoglobin from hemoglobin and hydrogen sulfide. They demonstrated that certain reducing substances, such as sodium hydrosulfite, phenylhydrazine, and hydrazine, acted as catalyzers for this reaction and suggested that clinical sulfhemoglobinemia might depend upon the presence of minute amounts of reducing substances in the blood. Later, Wallis (39) actually found reducing substances in the bloods of patients with sulfhemoglobinemia. In 1924, Vogel (40) reported sulfhemoglobinemia in a man whose job involved the production of p-aminophenol from nitrobenzene and who had been using sulfur pills. A reducing substance was found in the patient's serum which was not present in normal serum.

Snapper (41) stressed the importance of aniline derivatives in the production of sulfhemoglobinemia when he reported the presence of sulfhemoglobin in the blood of two patients who had taken large quantities of phenacetin. He demonstrated the formation of sulfhemoglobin in dogs when they were fed both sulfur and phenacetin at the same time. Neither sulfur nor phenacetin was active when fed separately. Snapper believed that phenacetin, or its derivative p-aminophenol, sensitized the red cells to the action of the sulfur. Ivens and van Vollenhoven (42) found that p-aminophenol but not phenacetin could accelerate the formation of sulfhemoglobin from hemoglobin and hydrogen sulfide *in vitro*. They assumed that phenacetin was metabolized by the body to p-aminophenol which was the active form of the drug.

A number of other reports of sulfhemoglobinemia after the ingestion of acetanilid, phenacetin, or nitrobenzene have appeared (43). One report by Van den Bergh and Revers (44) is interesting in that it demonstrated the appearance of sulfhemoglobin after the ingestion of pyridium, a precursor of sulfanilamide in the chemotherapy of bacterial infections. In all cases, sulfhemoglobin has been noted to persist for long periods after the offending

drug had been removed. This behavior is to be contrasted with that of methemoglobin and is probably due to the fact that hemoglobin cannot be reformed directly from sulfhemoglobin.

Archer and Discombe (8) have found that sulfanilamide can accelerate the formation of sulfhemoglobin from hemoglobin and ammonium sulfide *in vitro* but their work was done at unphysiological acidity and concentration. Paton and Eaton (6) were unable to demonstrate this effect, and we were likewise unsuccessful (unpublished experiments). By analogy to the situation in phenacetin poisoning, we therefore assume that sulfanilamide is converted by the body into some derivative which can accelerate sulfhemoglobin formation from the action of absorbed sulfide compounds on hemoglobin. It is possible that this active derivative is the same one which is responsible for methemoglobin formation.

If these assumptions are true, then the development of sulfhemoglobin after sulfanilamide ingestion depends upon (a) the rate of formation and destruction of the active agent, (b) the intensity of its catalyzing effect, (c) the presence of the necessary sulfide compounds, and (d) the rate of destruction of sulfhemoglobin. A direct relationship between the sulfhemoglobin and sulfanilamide concentrations in the blood would not occur since the factor of the presence of sulfides is quite independent and variable. For the same reason one would not expect a direct correlation between sulfhemoglobin and methemoglobin concentrations. However, there would be a greater tendency to develop sulfhemoglobinemia during long courses of the drug since it is obvious that the longer the accelerator is present in the blood stream, the greater chance there would be of having some peculiarity of intestinal function which would allow the admission of sulfides into the blood stream. Furthermore, since sulfhemoglobin is relatively stable in the body, minute amounts could be formed which would accumulate and become clinically demonstrable only after a period of time. All these deductions are in accord with the data presented.

Other blood pigments

In several blood samples it was noticed that there was a diffuse darkening of the entire blood

with particular absorption in the red region of the spectrum. The maximum of this absorption was at 670 $m\mu$. The plasma, however, was not abnormal so that the pigment was probably not hematin or methemalbumin. The absorption was not changed by reduction with hydrosulfite or by the addition of cyanide (remembering that the blue photo-oxidation product of sulfanilamide is destroyed by hydrosulfite). The pigment giving this absorption was unstable and faded in the course of several hours even if blood was kept at 0° C. A similar pigment has been noted by one of us (H. O. M.) in the blood of occasional patients treated with nicotinic acid or with bromides and acetanilid. These bloods did not contain methemoglobin or sulfhemoglobin. The nature of the pigment is unknown.

The etiology of sulfanilamide cyanosis

The question as to whether the cyanosis is entirely due to hemoglobin derivatives is not within the scope of this paper. Cyanosis cannot be quantitated and it depends on many variables besides the color of the blood. The difficulty becomes insurmountable when dealing with negroes who make up a large part of our series. Furthermore, the extent of methemoglobinemia or sulfhemoglobinemia necessary to produce cyanosis is not known. The consensus of opinion seems to be that a relatively large amount of methemoglobin must be present but Bensley, Rhea, and Mills (45) have recently reported two cases of idiopathic familial methemoglobinemia with cyanosis at times when the concentration of methemoglobin was quite low. In one of these cases cyanosis was present when the methemoglobin was only 0.7 gram per cent and the total pigment was 14.4 grams per cent. The per cent of total pigment in the form of methemoglobin was therefore less than 5 per cent, and the blood would appear normal on spectroscopic examination to all but the very trained observer. No other cause for cyanosis could be demonstrated in this case. Since the alpha sulfhemoglobin band is some three times as strong in intensity as that of methemoglobin, it would take far smaller quantities of sulfhemoglobin to produce cyanosis.

With these restrictions it is our impression however that the cyanosis does not parallel the

methemoglobin level in some cases. It may be that some product of sulfanilamide metabolism may stain the red cells—perhaps the breakdown products of the active agent which we have postulated. The important fact remains that the incidence of methemoglobinemia and sulfhemoglobinemia points to the appearance of a physiologically active substance and not to the metabolism of sulfanilamide directly into inactive colored compounds.

SUMMARY

There were 960 blood samples from 476 patients receiving sulfanilamide examined for free sulfanilamide content, methemoglobin, and sulfhemoglobin. In the 277 patients who had methemoglobinemia and in the 37 patients who had sulfhemoglobinemia at some time, as demonstrable by the hand spectroscope, quantitative spectrophotometric determinations were made.

The percentage of bloods which showed methemoglobin was highest in the group that had high sulfanilamide content. The average methemoglobin value of all bloods was proportional to the sulfanilamide concentration. Methemoglobinemia did not depend upon sex, but was somewhat more frequent and more pronounced in the very young. The average methemoglobin concentration tended to diminish with increasing duration of therapy at constant blood sulfanilamide levels up to 8 mgm per cent but at higher sulfanilamide concentrations there was a tendency for the methemoglobin to increase with time. After a single dose of sulfanilamide, the maximal methemoglobinemia occurred several hours after the blood sulfanilamide had reached its peak.

Sulfhemoglobinemia was more frequent after long courses of sulfanilamide, but did not bear any relationship to age, sex, or the concentration of sulfanilamide or methemoglobin in the blood.

On the basis of these findings, it is postulated that an active substance is normally produced in the course of sulfanilamide metabolism which causes the production of methemoglobin* and sulfhemoglobin. The statistics presented are found to agree with the concept that methemoglo-

* We have demonstrated the formation of such an active substance when surviving tissues react with sulfanilamide *in vitro*. These experiments will be published.

THE *IN VITRO* FORMATION OF AN OXIDIZING AGENT BY SURVIVING TISSUES AND SULFANILAMIDE

By J S HARRIS

(From the Departments of Biochemistry and Pediatrics Duke University School of Medicine Durham)

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Methemoglobin formation generally occurs during sulfanilamide therapy in humans (1, 2, 3, 4, 5) From the clinical statistics on methemoglobinemia it has been deduced that sulfanilamide is partially converted by the body into some active agent which can function as an oxidant (5) It is the purpose of this paper to determine whether the oxidation of hemoglobin is performed by sulfanilamide itself or whether the active agent is produced through the interaction of tissues and sulfanilamide

METHODS

Normal animals were killed by a blow on the head followed by decapitation The tissues were removed and slices of 0.2 to 0.4 mm thickness were immediately prepared These were suspended in 5 ml Ringer phosphate solution of pH 7.4 (6) containing 0.1 gram per cent glucose varying concentrations of sulfanilamide, and the saline washed erythrocytes from approximately 0.5 ml of normal human blood The mixtures were placed in 50 ml Erlenmeyer flasks and were shaken constantly in a water bath at 37.5° C for approximately 2 hours The tissue slices were removed dried and weighed The dry weight was generally between 30 and 50 mgm. The red cells were centrifuged, washed with physiological saline, hemolyzed with saponin, and diluted to 10 ml with 1/20 M pH 7.4 phosphate buffer Methemoglobin and total hemoglobin were determined spectrophotometrically by the change in extinction at 634 mμ upon conversion to cyanmethemoglobin (7) The presence of methemoglobin was confirmed by the disappearance of the alpha band upon the addition of cyanide hydrosulfite, and ammonium hydroxide Furthermore, the percentage of methemoglobin in the mixture as determined from the ratio of the extinctions at 575 mμ to 560 mμ (8) checked with the quantity as previously determined Only methemoglobin could give these results The bands of pigments other than met-

hemoglobin and oxyhemoglobin were not detected with the hand spectroscope.

EXPERIMENTAL DATA

The incubation of erythrocytes with Ringer-phosphate solution containing from 0 to 250 mgm. per cent of sulfanilamide never caused the conversion of more than 1 per cent of the total hemoglobin to methemoglobin The incubation of erythrocytes with tissue slices of the liver, kidney muscle, spleen of the mouse rabbit, rat, cat, and guinea pig was likewise without appreciable effect on the hemoglobin However, when liver slices were incubated with sulfanilamide and red cells, the formation of methemoglobin invariably occurred A sample protocol is shown in Table I Similar results were obtained with the livers of the cat guinea pig and rabbit Other animals were not tried These results are shown in Figure

TABLE I

The formation of methemoglobin upon incubation of liver slices with sulfanilamide and red cells*

Preparation Liver slice Hemoglobin added Human red cells (66 mgm of oxyhemoglobin in mouse experiment 87 mgm. of oxyhemoglobin in rat experiment) Duration of incubation 2 hours.

Animal	Final sulfanilamide concentration	Weight of dry tissue		Methemoglobin		Conversion of total hemoglobin
	mgm per cent	mgm.	mgm.	mgm per 100 mgm of dry tissue	per cent	
No tissue	100	0	0.5			0.8
Mouse	0	42	0.5			0.8
Mouse	25	31	12.0	0.4		18.2
Mouse	50	32	15.4	0.5		23.3
Rat	0	47	0.5			0.6
Rat	10	54	14.3	0.3		16.4
Rat	25	42	22.0	0.5		25.3
Rat	50	51	33.0	0.6		38.0
Rat	100	51	38.0	0.8		43.6

* Supernatant fluid after removal of tissue and red cells was colorless.

natant fluid was incubated for 45 minutes with red cells and the formation of methemoglobin resulted. The supernatant fluid from this reaction was again incubated with fresh red cells and methemoglobin was again formed. The results are seen in Table III-B.

These effects might occur because all of the oxidizing agent had not been utilized in an irreversible reaction by the first addition of red cells. However, a consideration of the rate of reaction shows that methemoglobin formation tends to reach a maximum at the end of 45 to 60 minutes even when high concentrations of sulfanilamide are used. This is shown in Figure 3 where the supernatant fluid from the mixture in Ringer-phosphate containing 100 mgm per cent of sulfanilamide was incubated with red cells for varying lengths of time. Nevertheless, if fresh red cells are incubated with the supernatant fluid (see Table III), an additional methemoglobin formation to an extent of 2/3 of the original intensity occurs. Furthermore, the supernatant fluid from the incubation of liver slices in a solution containing 100 mgm per cent of sulfanilamide caused the formation of 21 mgm of methemoglobin in 60 minutes in the presence of red cells having a total of 65 mgm of oxyhemoglobin. The same supernatant fluid, how-

ever, caused the production of 34 mgm of methemoglobin in the same period of time when incubated with double the quantity of red cells. The ratio of oxidized hemoglobin to total hemoglobin thus remains relatively the same. These results seem best to be explained by the action of a redox system on the hemoglobin-methemoglobin system.

The most intense methemoglobin formation per mgm of sulfanilamide occurred when liver slices were incubated with red cells in 5 ml of a solution containing 10 mgm per cent of sulfanilamide, 14 mgm of methemoglobin being formed. This quantity of sulfanilamide, if converted into a monovalent oxidizing agent, would be equivalent to 48.5 mgm of hemoglobin. Therefore 29 per cent of the theoretical yield was obtained. It is probable, however, that most of the sulfanilamide is not converted into an active agent. It has been found that the destruction of sulfanilamide by the surviving liver as tested by the method of Marshall (10) is negligible (9). Unless the amino group is held intact during the formation of the active agent, the substance must be autoxidizable and must act as a catalytic reversible redox system. Furthermore, when the supernatant fluid from the incubation of rat liver slices in sulfanilamide solutions is mixed with

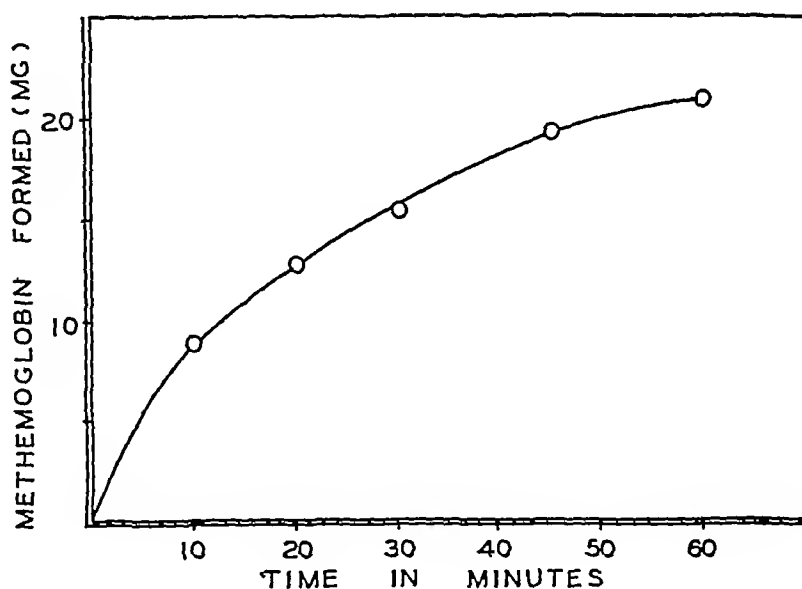


FIG 3 RATE OF FORMATION OF METHEMOGLOBIN IN RED BLOOD CELLS INCUBATED WITH THE SUPERNATANT FLUID OF THE REACTION OF RAT LIVER SLICES ON SULFANILAMIDE

red cells under anaerobic conditions, very little methemoglobin is formed as compared to the result of the same experiment under aerobic conditions. This suggests but does not prove that the continual reoxidation of the agent by air ordinarily occurs. These problems are being investigated more thoroughly.

It is possible that the active agent may owe its effect to an anticalase action. Accumulation of hydrogen peroxide during the metabolism of the red cells might result and might cause the oxidation of hemoglobin to methemoglobin. To eliminate this possibility, the following experiment was performed. Liver slices were incubated in Ringer phosphate solution containing 100 mgm per cent of sulfanilamide. The supernatant fluid after centrifugalization was incubated for 30 minutes with human red cells. The mixture was then hemolyzed with saponin. The same procedure was followed in another vessel save that the supernatant fluid of liver slices incubated in Ringer phosphate solution without sulfanilamide was used. To each mixture of hemolyzed cells and supernatant fluid was added a small amount of hydrogen peroxide and the oxygen evolution was measured manometrically in the Warburg apparatus. The rate and extent of the catalase action were found to be identical in both mixtures. The extent of methemoglobin formation in the preparation containing sulfanilamide was then compared with another similar preparation which had not been mixed with hydrogen peroxide. No change in the amount could be detected although both had much more methemoglobin than did the control without sulfanilamide. The entire experiment was then repeated with the exception that the red cells were not hemolyzed before the addition of the peroxide and the same results were obtained. If the active agent acted through anticalase action, the oxygen evolution after the addition of hydrogen peroxide should have been slower in the solutions containing sulfanilamide and an increased formation of methemoglobin should have resulted in those solutions when peroxide was added.

Using the technique of testing the supernatant fluids (from the incubation of tissue and sulfanilamide) with red cells it has been found that precipitation of the protein with trichloroacetic

acid does not remove the active substance. The agent is dialyzable through ordinary viscose membranes is relatively stable at 0°, and is destroyed by boiling. The substance can exert its action on red cells when they are suspended in serum.

DISCUSSION

The formation of an oxidizing agent from sulfanilamide by tissues adequately explains the methemoglobinemia which has been found in patients treated with this drug. Clinical methemoglobinemia has been noted to depend upon the concentration of sulfanilamide in the blood, and to reach a peak several hours after the sulfanilamide concentration of the blood had reached its peak (5). The methemoglobinemia of patients was also found to depend slightly upon the concentration of circulating hemoglobin. All these results have been repeated with the use of surviving tissues *in vitro*.

It is to be noted that all the supernatant fluids from the incubation of tissue with sulfanilamide were colorless. Nevertheless these preparations were more active in forming methemoglobin than some of the blue solution prepared by the action of ultraviolet light on sulfanilamide solutions by the method of Ottenberg and Fox (11). It is therefore very improbable that the active agent formed by tissues is identical with the blue photo-oxidation product. If this were true, the color should have been easily detected in the supernatant solutions. In addition a marked destruction of sulfanilamide (at least of the amino group) occurs during the action of ultraviolet light whereas practically no destruction can be detected by the same method when sulfanilamide is incubated with tissue.

Maun, Shinn and Mellon (12) have suggested that a substance having an anticalase action is formed by bacteria from sulfanilamide and that the resultant accumulation of hydrogen peroxide may explain the therapeutic action of the drug. The formation of methemoglobin by tissues and sulfanilamide does not depend upon a similar action and the agent reported herein is not identical with the anticalactic substance because (a) the red cells after incubation with the agent retain their catalase activity (b) the oxygen uptake of red cells in the absence of an autooxidizable carrier is so small that, even

were transformed to hydrogen peroxide, not more than 1 mgm of methemoglobin would have been formed under the conditions of our experiments, (c) the activity of the agent persisted after preliminary interaction of red cells, which should have absorbed any anticatalase present

Rimington (13) has suggested that the agent causing methemoglobinemia may also account for the excess porphyrin excretion seen during sulfanilamide therapy. The proof of this possibility and the relation of the agent to other toxic manifestations must await the isolation of larger quantities of the active agent

The oxidizing agent may account for some of the therapeutic properties of the drug. Levaditi and Vaisman (14) have shown that sulfanilamide which has been absorbed *via* the gastro-intestinal tract seems to be more potent than locally applied sulfanilamide, and they believed that the gastro-intestinal tract or viscera caused some change in the drug. It is planned to study the bactericidal activity of the agent²

SUMMARY

It has been demonstrated that sulfanilamide cannot function as an oxidizing agent on hemoglobin. Upon the interaction of certain tissues with sulfanilamide, an oxidizing agent is formed which can cause the production of methemoglobin. This process has been discussed in relation to the clinical findings in patients on sulfanilamide therapy.

BIBLIOGRAPHY

1 Wendel, W B., Methemoglobin determination, clinical method. *J Lab and Clin. Med.*, 1938, 24, 96

² Since this paper was accepted for publication, the theoretical importance of oxidation products of sulfanilamide with high oxidizing potentials has been admirably stressed by P A Shaffer (The mode of action of sulphanilamid. *Science*, 1939, 89, 547)

- 2 Evelyn, K. A., and Malloy, H T, Microdetermination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood *J Biol. Chem.*, 1938, 126, 655
- 3 Hartmann, A. F., Perley, A. M., and Barnett, H L., A study of some of the physiological effects of sulfanilamide, methemoglobin formation and its control. *J Clin Invest.*, 1938, 17, 699
- 4 Lockwood, J S., Coburn, A F., and Stokinger, H E., Studies on mechanism of action of sulfanilamide, bearing of character of lesion on effectiveness of drug *J A M A*, 1938, 111, 2259
- 5 Harris, J S., and Michel, H O, The formation of methemoglobin and sulfhemoglobin during sulfanilamide therapy *J Clin Invest.*, 1939, 18, 507
- 6 Van Heyningen, W E., Inhibition of respiration by cyanide. *Biochem J*, 1935, 29, 2036
- 7 Michel, H O., and Harris, J S. (To be published)
- 8 Michel, H O., A spectrophotometric method for the determination of methemoglobin in hemoglobin solutions *J Biol Chem. (Proc.)*, 1937, 119, lxi.
- 9 Klein, J R., and Harris, J S., Acetylation of sulfanilamide *in vitro* *J Biol Chem.*, 1938, 124, 613
- 10 Marshall, E K., Jr., Determination of sulfanilamide in blood and urine. *J Biol Chem.*, 1937, 122, 263
- 11 Ottenberg, R., and Fox, C. L., Jr., Explanation for cyanosis of sulphanilamide therapy *Proc. Soc. Exper Biol and Med*, 1938, 38, 479
- 12 Main, E. R., Shinn, L. E., and Mellon, R. R., Anticatalase activity of sulfanilamide and related compounds I Effect of ultraviolet irradiation. *Proc. Soc. Exper Biol. and Med.*, 1938, 39, 272
- 12a Shinn, L. E., Main, E. R., and Mellon, R. R., Anticatalase activity of sulfanilamide and related compounds II Relation to growth inhibition in pneumococcus *Proc. Soc. Exper Biol and Med.*, 1939, 39, 591
- 13 Rimington, C., Disturbances of pigment metabolism following administration of drugs of the sulphonamide series and simpler related substances *Proc. Roy Soc. Med.*, 1939, 32, 351
- 14 Levaditi, C., and Vaisman, A., Au sujet du mécanisme de l'action thérapeutique des dérivés benzéniques sulfurés dans l'infection streptococcique expérimentale. *Compt. rend. Soc. de biol.*, 1938, 128, 476

CLINICAL STUDIES OF THE BLOOD VOLUME. VII CHANGES IN
BLOOD VOLUME IN BRIGHT'S DISEASE WITH OR WITHOUT
EDEMA, RENAL INSUFFICIENCY, OR CONGESTIVE
HEART FAILURE, AND IN HYPERTENSION¹

BY ALFRED W HARRIS AND JOHN G GIBSON 2D

(From the Medical Clinic of the Peter Bent Brigham Hospital and the Department of
Medicine Harvard Medical School Boston)

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Richard Bright (1) in 1836 remarked upon the watery condition of the blood in the edematous phase of disease of the kidneys and attributed it to a loss of albuminous substances into the urine with a resultant thinning of the blood. Since that time numerous investigators, aware of the role of the kidneys in the excretion of water and electrolytes and of the relation of the blood protein concentration to edema, have studied the water balance in Bright's disease, many with particular reference to the total amount of circulating blood and the changes in the plasma and total blood volume which occur during the edematous stage of renal disease and during diuresis.

The evaluation of the few reports in the literature on the state of the blood volume in Bright's disease is difficult because of the variety of methods of determining the plasma volume employed, the wide divergence in normal values obtained by these methods and the lack of a consistent clinical classification of the cases of nephritis studied.

(Several authors give no further description than that their cases had "renal edema. Thus Plesch (2) using a CO method and Schmidt (3) using a Congo Red dye method of determining the blood volume found total blood volume above normal while Waterfield (4) using a CO method found it decreased, and Linder *et al* (5) reported plasma volume as determined by a dye method to be normal in cases with 'renal edema.)

(Hartwich and May (6) and Litzner (7) both of whom used dye methods found a normal plasma but high blood volume, whereas Rusznayak (8) with a dye method found total blood volume

to be subnormal due to a lowered red cell volume. In chronic glomerular nephritis Brown and Rowntree (9) using the original dye method, reported plasma volume to be normal and total blood volume decreased in 12 cases, but Hartwich and May (6) reported 6 cases with increased and 4 with decreased total blood volumes)

Darrow (10) reported 2 cases of "nephrosis" studied by a dye method with subnormal total blood volumes while Brown and Rowntree (9) found total blood volume normal, if no anemia was present, and above normal in their anemic cases. Waterfield (4) stated that in the edematous stage of renal disease total blood volume was least when the amount of edema was greatest and rose during diuresis due to an increase in plasma volume, this latter finding being in agreement with that of Darrow (10). Brown and Rowntree (9) concluded that the mechanism of diuresis was different in chronic nephritis than in "nephrosis," since in the former condition total blood volume decreased but no constant change in either plasma or red cell volume was observed in the latter during diuresis.

[Rusznayak (8) reported very low total blood volume in 24 cases of "extra renal" hypertension. He also made the interesting observation that only in a group of cases with 'contracted kidneys' was the total blood volume ever greater than normal.

] It is apparent that the available information concerning the state of the blood volume in hypertension and nephritis is of a contradictory nature, and we, therefore considered it worth while to re investigate the subject using a method (11) of determining the blood volume which we have found to minimize the effects of errors inherent in many of the techniques employed by the above authors.

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MATERIALS STUDIED

Cases studied were selected from the Medical Service and Out-Door Department of the Peter Bent Brigham Hospital. For analytical purposes Christian's (12) clinical classification of nephritis was used, patients having been divided into 5 groups as follows

Group I Seven males and 6 females with chronic glomerular nephritis without renal edema. Characteristic of this group was persistent albuminuria and cylindruria and varying degrees of anemia, hypertension, impaired renal excretion of phenolsulphonephthalein and blood nonprotein nitrogen retention

Group II Two males and 2 females with acute hemorrhagic nephritis. All of these cases had albuminuria, hematuria, and anemia

Group III Nine males and 1 female with sub-acute glomerular nephritis with renal edema (nephrosis syndrome). All of these cases had profuse albuminuria, doubly refractile lipid bodies in their urine, high blood cholesterol, low basal metabolic rates, and varying degrees of elevation in systolic and diastolic blood pressure and of hypoproteinemia with distortion of the normal albumin-globulin ratio. In 6 of these cases the course of the blood volume was followed by repeated determinations during the course of diuresis

Group IV A mixed group, who presented in addition to renal disease with or without renal failure the common finding of congestive heart failure, consisting of 1 female with bilateral polycystic kidneys (Case 368), 1 male and 2 females with chronic glomerular nephritis (Cases 359, 380, and 403), and 1 male and 1 female with chronic vascular nephritis (Cases 393 and 374)

Group V Five males and 11 females with essential hypertension. In all cases systolic blood pressure was 160 mm of mercury or above, and in none was there evidence of renal insufficiency or congestive heart failure

METHODS

Plasma, circulating red cell, and total blood volume were determined by the dye method of Gibson and Evans (11), venous pressure by the direct method of Evans (13), circulation time by the intravenous decholin method of Winternitz,

Deutsch, and Brull (14), hemoglobin by the Osgood-Haskins modification of the method of Sahli (15), blood urea nitrogen by the urease method of Van Slyke and Cullen (16), protein and nonprotein nitrogen concentration by modifications of the methods of Folin (17) and Koch and McMeekin (18)

RESULTS

Height was taken as the basis for prediction of normal volume, since surface area as a basis would have given a distorted normal value in those patients who had extensive edema and in those who were cachectic in the non-edematous stages of their chronic disease

Plasma, circulating red cell and total blood volume, blood chemistry, hematology, and hemodynamics in Groups I, II, III, and IV are shown in Table I and the corresponding findings in Group V in Table II

The distribution of cases in Groups I, III, and V in terms of percentage deviation from predicted normal plasma, circulating red cell and total blood volume is shown in Figure 1. The normal range of plasma, circulating red cell, or total blood volume may be considered to be plus or minus 15 per cent (19). The statistical method of determining the standards of normality employed in this study is such that the average values in terms of percentage deviation from normal for any group of individuals with essentially normal plasma and total blood volumes will closely approximate zero. Therefore, in abnormal groups, the averages of deviations from normal represent significant departures in plasma, circulating red cell, and total blood volume from the normal state characteristic of the common diseased state of the members of the group, even though the average values themselves may fall within the accepted range of normality. It is, therefore, evident from the average values that the hypertensives constitute a group with plasma, circulating red cell, and total blood volumes well within the range of normality. In Groups I and III total blood volume is definitely subnormal, more so in the latter than in the former groups, due to a great reduction in circulating red cell volume, which in both groups is considerable enough to more than offset a higher than normal plasma volume

TABLE I

Blood volumes hemodynamics and blood chemistry in nephritis with and without renal edema and congestive heart failure

Case number	Date	Sex	Age	Height	Weight	Red cell count	Hemoglobin	Blood urea nitrogen	Non protein nitrogen	Serum Proteins			Blood pressure	Venous pressure	Creatinine time	Plasma volume	Percentage derived from normal	Red cell volume	Percentage derived from normal	Total blood volume	Percentage derived from normal
										Total	Albumin	Globulin									
			years	cm.	lbs.	mill. Hg.	per cent of cells	mgm. per 100 cc.	mgm. per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	mm. Hg.	mm. Hg.	mgm. per 100 cc.	cc.	cc.	cc.	cc.	cc.	cc.
GROUP I—18 CASES WITH CHRONIC GLOMERULAR NEPHRITIS WITHOUT EDEMA																					
337	June 24, 1933	M	37	165.0	52.3	4.01	40.0	37	6.8	3.3	3.3	184/112	90	13	2550	-4.3	1770	-18.8	4450	-11.0	
338	June 25, 1933	F	33	172.9	60.0	3.52	37.4	40	6.6	3.8	3.1	130/90	110	17	2190	-37.1	1300	-37.9	4390	-5.3	
341	June 27, 1933	F	37	154.8	50.6	4.40	38.0	30	6.1	2.7	2.9	130/100	150(7)	13	2300	-16.3	1490	-6.8	4280	-8.5	
342	June 28, 1933	F	33	170.0	63.2	5.00	43.8	6	6.6	4.6	2.0	108/90	120	9	3480	-16.3	2370	-0.0	4850	-0.3	
365	August 30, 1933	M	31	173.3	72.0	3.13	34.4	50	4.0	3.4	2.5	140/90	90	13	2723	-12.5	1420	-41.8	4145	-23.3	
371	September 13, 1933	M	33	167.6	52.5	4.25	34.3	49	5.7	7.1	4.3	235/70	80	13	3490	-20.0	1910	-14.0	4790	-2.3	
372	September 14, 1933	F	40	163.5	62.7	3.57	39.4	36	5.7	5.6	3.1	160/100	120	13	2440	-6.3	1110	-31.0	3750	-7.0	
373	September 18, 1933	M	37	167.5	52.5	4.0	41.6	37	6.3	4.4	2.2	123/93	90	9	2330	-19.6	1740	-22.6	4070	-21.3	
375	September 25, 1933	F	19	162.5	51.4	4.33	37.3	10	7.7	4.6	3.1	134/85	105	10	2130	-11.7	1260	-21.3	3330	-14.5	
379	October 3, 1933	F	25	153.0	56.0	4.24	43.1	10	6.5	3.7	2.9	134/88	105	13	3000	-3.3	1530	-3.3	3260	-0.6	
382	February 15, 1939	M	34	153.0	73.5	2.45	36.3	61	11.4	6.6	4.5	2.1	220/130	145(7)	24	5070	-64.3	1900	-60.3	6370	-8.4
394	February 16, 1939	F	43	154.8	72.5	3.67	37.7	39	5.9	4.2	2.8	200/110	45	18	3740	-56.8	810	-44.8	4550	-16.7	
404	March 7, 1939	M	31	169.4	65.8	3.48	39.5	29	5.9	3.4	2.1	140/70	150	13	3010	-2.3	1370	-16.9	4390	-18.9	
GROUP II—6 CASES WITH ACUTE HEMORRAGIC NEPHRITIS																					
341	March 23, 1935	F	16	157.5	47.5	4.40	34.8	16	5.8	3.4	2.2	115/75	8	1600	-37.3	900	-41.5	3290	-0.3		
350	September 9, 1935	F	14	165.0	40.8	3.37	35.7	6	7.5	4.0	3.5	108/78	2430	-8.8	1370	-11.3	2370	-1.0			
401	February 23, 1939	M	14	167.6	50.0	2.30	43.1	20	6.1	2.3	2.8	139/72	2390	-9.1	1490	-24.0	4370	-15.9			
403	March 16, 1939	M	61	174.0	49.4	3.50	33.8	33	3.9	2.0	1.9	103/72	2300	-9.1	1400	-42.1	4300	-23.6			
GROUP III—10 CASES WITH SUBACUTE GLOMERULAR NEPHRITIS WITH EDEMA (NEPHROSIS STREPTOCOCCICA)																					
8A	December 6, 1934	M	43	168.0	76.6	2.80	44.1	33	6.5	3.3	3.3	200/190	140	15	3170	-7.5	1710	-17.3	5230	-10.7	
2B	October 10, 1935	M	43	163.9	73.0	3.31	37.3	39	6.3	3.5	2.8	180/100	100	10	3740	-7.3	1400	-39.3	5140	-2.1	
192	June 3, 1935	M	78	166.0	55.9	6.05	40.0	41	3.3	4.5	1.5	2.0	100/40(7)	10	2150	-17.8	1110	-30.1	3260	-37.4	
290A	February 18, 1937	M	17	183.3	73.4	3.11	31.1	11	3.3	2.5	2.3	160/110	110	16	2420	-3.3	1250	-40.0	4970	-18.4	
300B	February 20, 1937	M	17	183.3	69.0	3.33	33.7	23	3.4	2.4	1.1	154/85	90	16	2810	-9.4	1810	-39.7	5220	-11.1	
390C	March 4, 1937	M	17	183.3	69.0	3.33	33.8	40	5.6	3.0	2.6	159/95	105	9	2425	-3.9	1260	-35.0	3195	-12.3	
390D	March 13, 1937	M	17	183.3	71.3	2.87	39.8	31	3.1	3.4	1.8	150/140	10	3303	-6.5	1673	-38.3	5183	-11.8		
293E	October 4, 1935	M	17	183.3	67.0	2.87	33.1	53	6.5	2.3	2.8	170/110	10	4530	-41.4	1340	-48.3	4990	-2.0		
295	February 29, 1937	M	14	180.0	43.0	4.30	40.9	14	3.4	1.8	1.5	143/125	70	11	1970	-33.7	1150	-41.5	3325	-47.3	
297A	March 10, 1937	M	20	172.5	60.3	3.33	33.3	31	3.8	2.3	1.3	144/92	130	10	3470	-13.8	1850	-31.3	5132	-6.1	
297B	October 7, 1937	M	20	172.5	61.3	3.65	31.5	34	4.0	2.6	2.4	145/90	130	3370	-10.5	1430	-32.4	4920	-9.7		
214A	December 4, 1937	M	47	184.4	80.5	3.05	33.3	42	4.4	0.9	2.5	160/88	30	3170	-17.4	1890	-21.1	4790	-2.7		
214B	April 12, 1938	M	47	184.4	83.4	3.24	33.0	42	4.9	2.3	1.3	152/105	33	3870	-10.3	1690	-39.3	5290	-11.8		
324A	February 15, 1938	M	19	171.4	37.4	2.30	33.5	34	3.9	2.6	1.8	110/125	3170	-8.0	880	-38.5	4140	-23.0			
324B	February 24, 1938	M	19	171.4	41.4	3.90	33.0	35	3.8	1.7	1.9	130/130	3360	-11.8	940	-60.3	4500	-22.0			
324C	March 14, 1938	M	19	171.4	45.5	2.48	31.1	35	5.0	2.7	1.6	131/110	19	3390	-19.6	1770	-39.0	4570	-12.0		
324D	April 2, 1938	M	19	171.4	45.4	3.80	31.0	35	5.4	2.3	2.3	144/103	90	12	3290	-9.3	970	-91.0	4170	-21.6	
324E	May 11, 1938	M	19	171.4	45.8	3.10	33.6	42	6.4	2.9	2.3	154/131	31	2100	-3.9	1130	-52.3	4750	-21.3		
329	March 18, 1938	F	30	153.0	47.5	2.65	37.7	9	5.3	2.9	2.0	190/140	134	1800	-12.6	760	-49.9	3740	-4.8		
340	June 27, 1938	M	34	166.3	48.0	4.91	41.1	8	3.4	5.6	2.7	3.1	112/84	105	19	2280	-0.4	1170	-11.5	3530	-7.3
347A	September 27, 1938	M	37	179.5	63.0	4.01	50.8	3	3.1	2.6	1.6	110/70	16	2590	-18.8	2090	-7.0	4390	-7.6		
347B	September 29, 1938	M	37	179.5	63.4	4.91	53.3	3	3.3	2.3	1.6	120/80	15	2490	-23.3	2320	-12.8	5300	-7.0		
347C	February 3, 1939	M	37	179.5	65.0	5.93	45.8	31	6.4	4.8	1.6	103/70	17	2250	-2.2	2740	-6.3	6000	-8.3		
GROUP IV—6 CASES WITH NEPHRITIS AND CONGESTIVE HEART FAILURE																					
359	June 25, 1938	M	42	170.0	60.1	5.30	33.9	78	6.7	3.5	3.4	190/190	150	23	4000	-34.7	2550	-9.8	6550	-23.5	
363A	September 8, 1938	F	46	156.3	71.4	3.03	33.3	78	6.6	3.4	3.2	180/80	130	17	3230	-40.3	990	-35.9	4390	-8.8	
363B	September 27, 1938	F	42	156.3	63.2	3.21	30.5	111	6.8	3.4	3.2	170/118	80	12	3950	-28.6	780	-40.3	3710	-8.0	
374	September 25, 1939	F	51	161.0	67.3	4.30	40.7	19	7.8	4.8	2.3	172/118	80	16	3130	-2.3	1720	-8.3	4230	-4.4	
290	October 20, 1939	F	39	161.8	50.8	2.56	22.3	26	5.9	3.3	2.5	174/104	135	19	3200	-23.3	950	-33.0	4190	-5.3	
322	February 10, 1939	M	50	167.6	63.4	6.19	43.4	4	7.4	5.9	3.8	210/100	135	18	2200	-10.3	2450	-7.7	4350	-8.3	
403	March 4, 1939	F	54	166.1	53.0	8.06	43.1	13	3.1	7.0	2.3	117/140	130	12	3440	-0.4	1350	-14.3	4390	-5.9	

TABLE II

Blood volume and hemodynamics in 16 cases of essential hypertension without renal involvement

Case number	Date	Sex	Age	Height	Weight	Blood pressure	Venous pressure	Circulation time	Plasma volume	Percent age deviation from normal	Red cell volume	Percent age deviation from normal	Total blood volume	Percent age deviation from normal	Hematocrit
			years	cm	kgm	mm Hg	mm H ₂ O	seconds	cc.		cc.		cc.		per cent of cells
37	June 13, 1935	F	67	158.8	59.2	238/100	65	12½	2200	-5.8	1370	-10.3	3630	-6.3	39.4
48	June 26, 1935	M		176.5	42.0	220/120	80	20	2570	-17.6	1900	-22.6	4470	-19.8	42.6
53	July 2, 1935	M		179.1	81.8	198/138	20	17	3120	-2.2	2300	-8.4	5420	-4.9	42.4
57	July 4, 1935	F		160.0	46.4	185/78	55	14	1680	-28.5	1320	-16.2	3000	-23.7	43.7
144	January 21, 1936	M	53	170.3	73.0		85		3260	+9.4	1830	-22.0	5090	-4.4	35.9
236	October 3, 1936	F	48	157.5	79.6	245/140	80	22	2360	+2.2	1540		3900	-1.3	39.6
242	November 3, 1936	F	37	158.8	58.0	240/130	85	14	2335		1365	-11.7	3700	-4.5	36.9
321	December 7, 1937	F	35	183.6	54.3	250/150		15	2130	-4.0	1540	+4.0	3670	-0.8	42.0
323	December 7, 1937	F	57	155.6	62.3	250/120			2470	+8.8	1810	+20.2	4280	+13.4	42.3
325	December 16, 1937	F	69	162.4	64.2	240/130	80	16	2555	+6.6	1575	-15.6	4130	+3.3	38.4
326	December 16, 1937	M	31	162.5	68.2	170/135	60	18	2560	-4.5	2750	+31.3	5310	+11.2	51.9
328	December 21, 1937	F	53	156.3	62.7	190/110	50	17	2610	+14.5	1860	+22.3	4470	+17.6	41.7
330	December 27, 1937	F	48	157.0	68.6	190/110	50	15	2300	+0.4	1620	+5.2	3920	+1.8	41.2
334	January 28, 1938	F	51	147.0	58.8	160/96		15	2090	+12.4	1080	-12.9	3170	+2.3	34.1
335	February 4, 1938	M	41	165.0		210/140			2310	-17.5	2060	-6.4	4370	-12.6	47.1
337	February 16, 1938	F	28	161.0	51.5	195/120		14	2820	+19.5	1500	-4.2	4320	+10.0	34.5

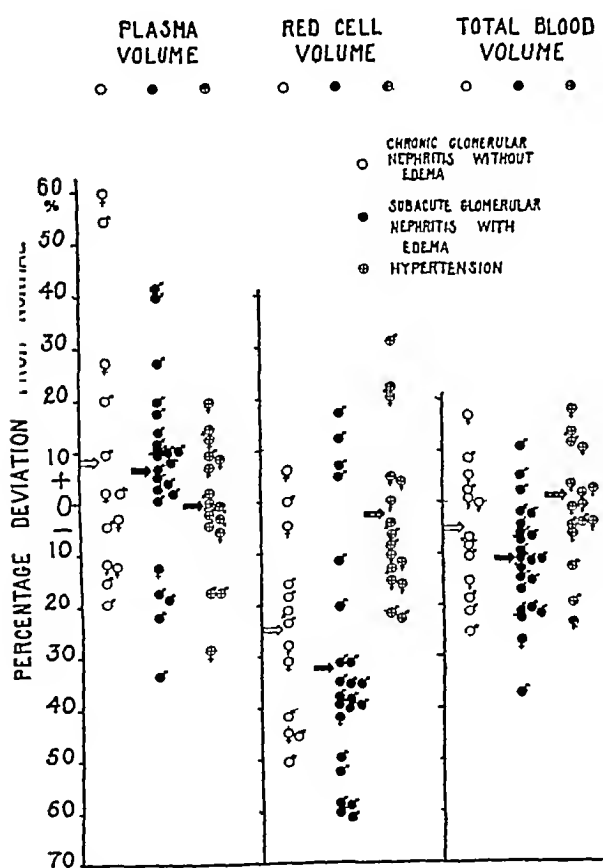


FIG 1 BLOOD VOLUME IN BRIGHT'S DISEASE

Case distribution in terms of percentage deviation from normal. Arrows indicate group averages. The hypertensives constitute a group with plasma, circulating red

The 4 cases with acute hemorrhagic nephritis (Group II) have subnormal circulating red cell and total blood volumes, the degree of reduction being comparable to that of Groups I and III

The relationship of the degree of abnormality in plasma, circulating red cell, and total blood volume to the red blood cell count in Groups I to IV inclusive is shown in Figure 2. The values for Groups I, II, and III were averaged together. A definite tendency is evident for the plasma volume to increase and attain higher than normal levels and for the circulating red cell volume to decrease further below normal as the red blood cell count declines. The interrelation of these changes is such that total blood volume remains below normal in about the same degree regardless of the erythrocyte level.

Changes in the plasma, circulating red cell, and total blood volume taking place in 6 cases of subacute glomerular nephritis with edema during periods of diuresis are shown in relation to changes in body weight in Figure 3. No consistent relationship between changes in the amount

cell, and total blood volume within normal limits. In subacute and chronic nephritis the great reduction in circulating red cell volume more than offsets the elevation in plasma volume so that total blood volume is below normal, and these deviations from normal are more extreme in the edematous than in the non-edematous phases of the disease.

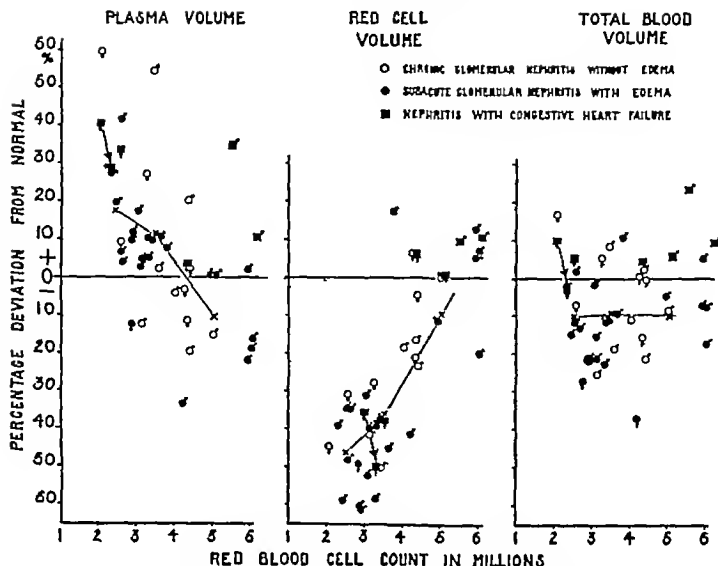


FIG. 2. BLOOD VOLUME IN RELATION TO ERYTHROCYTE LEVEL

The solid lines represent the average values for nephritics without congestive heart failure. As anemia develops in nephritis there is a progressive rise in plasma volume above and a fall in circulating red cell volume to below normal, with little change in total blood volume. The values for the cases with congestive heart failure are definitely higher than the average values for nephritics without congestive heart failure. The broken line indicates the change in volume accompanying compensation in a case with congestive heart failure.

of edema present as evidenced by body weight and the course of either plasma or circulating red cell volume, was observed. Thus, during the period of diuresis plasma volume increased in Case 8 fluctuated with a general tendency to increase, in Cases 290, 336, and 367 and decreased slightly in Case 297. In Case 324 during a period in which edema and body weight increased plasma volume fell. Circulating red cell volume underwent only minor changes in Cases 290, 336, and 367 in which plasma volume rose and in Case 297 in which plasma volume fell slightly but decreased considerably in Cases 8 and 324 this change being accompanied by an increase in plasma volume in the former and a decrease in the latter case.

Figure 4 shows the circulation time in Groups I, II, III, and IV in relation to the red cell count

While the majority of determinations were within normal limits the average trend did not indicate an acceleration of blood flow as anemia progressed, but rather the opposite condition. Values for cases in Group IV (with congestive heart failure) were definitely above the average for the other groups.

The degree of abnormality in plasma circulating red cell and total blood volume found in the 6 cases in whom renal disease was accompanied by congestive heart failure (Group IV) are shown in Figure 2 in relation to similar findings in the groups without cardiac insufficiency. The significance of these results will be considered in the discussion.

DISCUSSION

A certain degree of individual variation from the averages exhibited by the various groups as

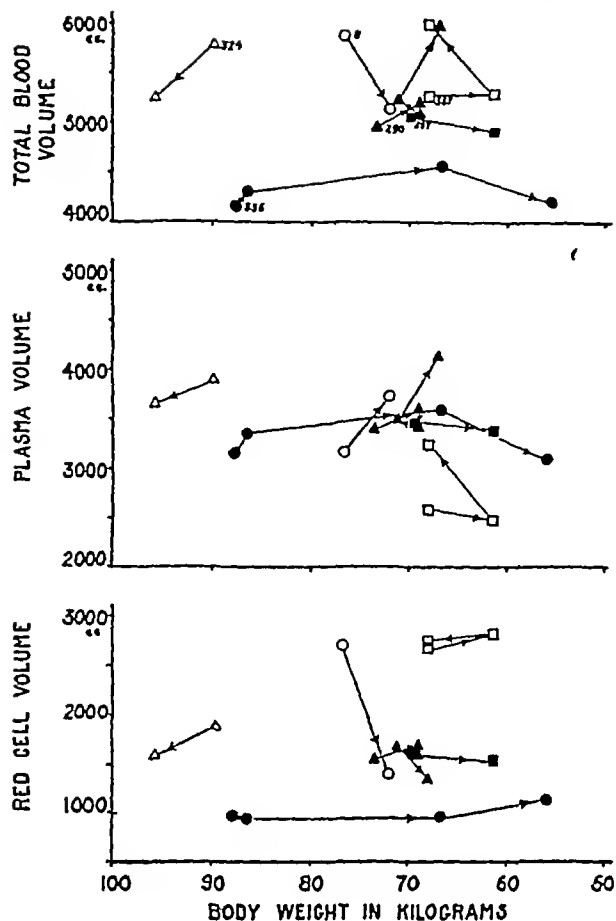


FIG 3 BLOOD VOLUME CHANGES DURING DIURESIS

Each symbol represents a case of subacute glomerular nephritis with edema (nephrosis syndrome) and corresponds to case numbers listed in Table I as follows: Case 8 = \circ , Case 290 = \blacktriangle , Case 297 = \blacksquare , Case 324 = \triangle , Case 336 = \bullet , and Case 367 = \square . The direction and degree of the changes in relation to changes in serum albumin, blood urea nitrogen, and erythrocyte level are discussed in the text.

a whole was encountered in this study. It must be remembered that the basis for prediction of individual normal blood volume, namely height, is of necessity an arbitrary one, and it may well be that the predicted normal volume was too high in some cases due to the cachexia of a wasting disease and too low in other cases who were in a fair state of nutrition when first studied. However, the average values of the groups as a whole represent deviations from the normal state which are truly characteristic of the disease under discussion.

Our findings are not in entire agreement with the views expressed by other investigators. The nature and magnitude of errors inherent in the early dye techniques, particularly those apt to be encountered in patients with disturbed circulations, have been commented on before (11) and may explain to some extent the general inconsistencies encountered in the literature. It would seem that the findings of a normal plasma volume (5, 9) and increased total blood volume (6) in chronic glomerular nephritis without renal edema are in error. We also differ from Brown and Rowntree (9), who stated that in "nephrosis" (in our terminology subacute glomerular nephritis with renal edema) total blood volume was normal when the disease was uncomplicated by anemia, and higher than normal when anemia was present. The majority of our cases had subnormal total blood volumes, and as the anemia progressed, further diminution occurred. We cannot agree with Brown and Rowntree (9) in their statement that in "nephrosis" loss of edema is accompanied

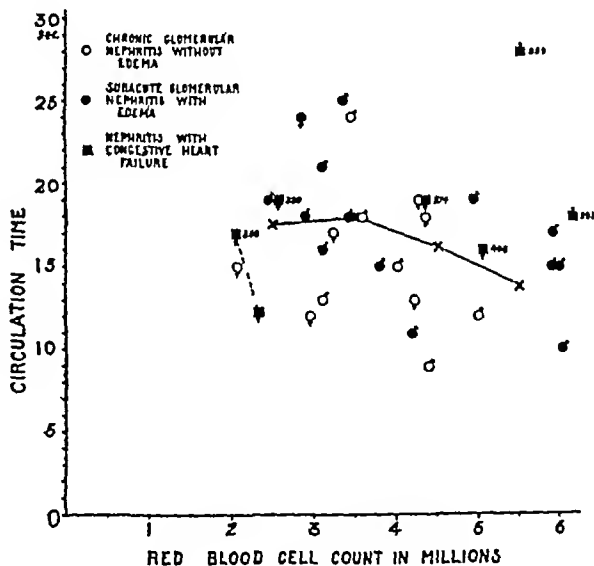


FIG. 4 CIRCULATION TIME IN BRIGHT'S DISEASE

As anemia progresses circulation time does not become faster as it does in primary and secondary anemia, and tends to be slower in the edematous than non-edematous cases. The average trend in both types of nephritis is indicated by the solid line. In congestive heart failure circulation time is slower than in nephritis without cardiac insufficiency regardless of the degree of anemia present. The broken line indicates the change in circulation time in Case 368 during recovery from congestive heart failure.

by no significant changes in either plasma or red cell volume as in our cases large changes in these blood volume components were observed during periods of diuresis.

In our opinion it may be stated definitely that in subacute or chronic glomerular nephritis with or without renal edema or renal insufficiency but without congestive heart failure the plasma volume is above and the circulating red cell and total blood volume are below a normal value for that individual in a state of good health. The diminution in circulating red cell and total blood volume tends to be more severe in subacute glomerular nephritis with renal edema (nephrosis syndrome) than in glomerular nephritis without edema.

There is a tendency towards a linear relationship between the degree of elevation above normal in the plasma volume and of decrease below normal in circulating red cell volume and the red cell count in nephritis. While this relationship is not numerically identical with that found in primary (20) and secondary anemia (21), it is similar in many respects. A distinct difference in respect to total blood volume exists, however, in that in primary anemia as the red cell count rises to 5 million under liver therapy the total blood volume increases to normal, whereas in Bright's disease, total blood volume remains below normal regardless of the erythrocyte level. The reason for this difference is not clear from the data obtained in this study and merits further investigation. With this exception in respect to the state of the blood volume the anemia of Bright's disease presents a striking similarity to primary and secondary anemia.

Of particular interest is the relationship of the course of the plasma volume to alterations in other factors known to affect body water regulation. That these factors may alter the plasma volume in opposite direction and varying degree is strongly suggested by the data presented in Figure 3 in which it is apparent that in an individual case the loss of a given amount of edema fluid is not accompanied by a constant change in plasma volume.

Our data permit us to examine the influence of 3 factors upon the plasma volume, namely the serum albumin concentration, the blood urea nitrogen concentration, and the degree of anemia

present as expressed by the red cell count. In Figure 5 the percentage abnormality in plasma volume is shown in relation to the serum albumin concentration. The average values indicate a tendency for plasma volume to increase as serum albumin increases. An identical relationship was found to exist between the plasma volume and the total serum protein concentration. In Figure 5 a similar tendency towards a direct relationship between plasma volume and blood urea nitrogen is illustrated. The increase in plasma volume accompanying a declining red cell count in both primary and secondary anemia has been commented on above and is illustrated with respect to the cases of nephritis herein studied in Figure 2.

The effect of these 3 factors on the course of the plasma volume in the 6 edematous cases followed during diuresis is summarized in Table III.

TABLE III
Theoretical and observed effect of changes in serum albumin concentration, blood urea nitrogen, and red cell count on plasma volume during diuresis

Case number	Serum albumin		Blood urea nitrogen		Red cell count		Observed change in plasma volume
	Observed change	Predicted effect	Observed change	Predicted effect	Observed change	Predicted effect	
8	Decreased	+	Increased	+	Decreased	+	+ 870
270	Fluctuated, then increased	+	Increased	+	Decreased	+	+1120
297	Slightly increased	+	Slightly increased	+	Slightly decreased	+	- 90
324	Increased	+	"	-	Increased	-	- 210
336	Fluctuated, then increased	+	Increased markedly	+	Decreased, then increased	±	+440, later - 60
357	Greatly increased	+	No change	0	No change	0	+ 870

* Data on blood urea nitrogen not available. Nonprotein nitrogen decreased slightly.

The conformity of the observed change in plasma volume to the change predicted on the basis of the sum total effect of variations in the 3 factors is striking.

This concept of the interrelationships of 3 factors regulating the balance between plasma and extravascular fluids is in keeping with the views expressed in the literature. Since the clinical application by Epstein (22) of Starling's work (23) on the osmotic pressure of the plasma proteins

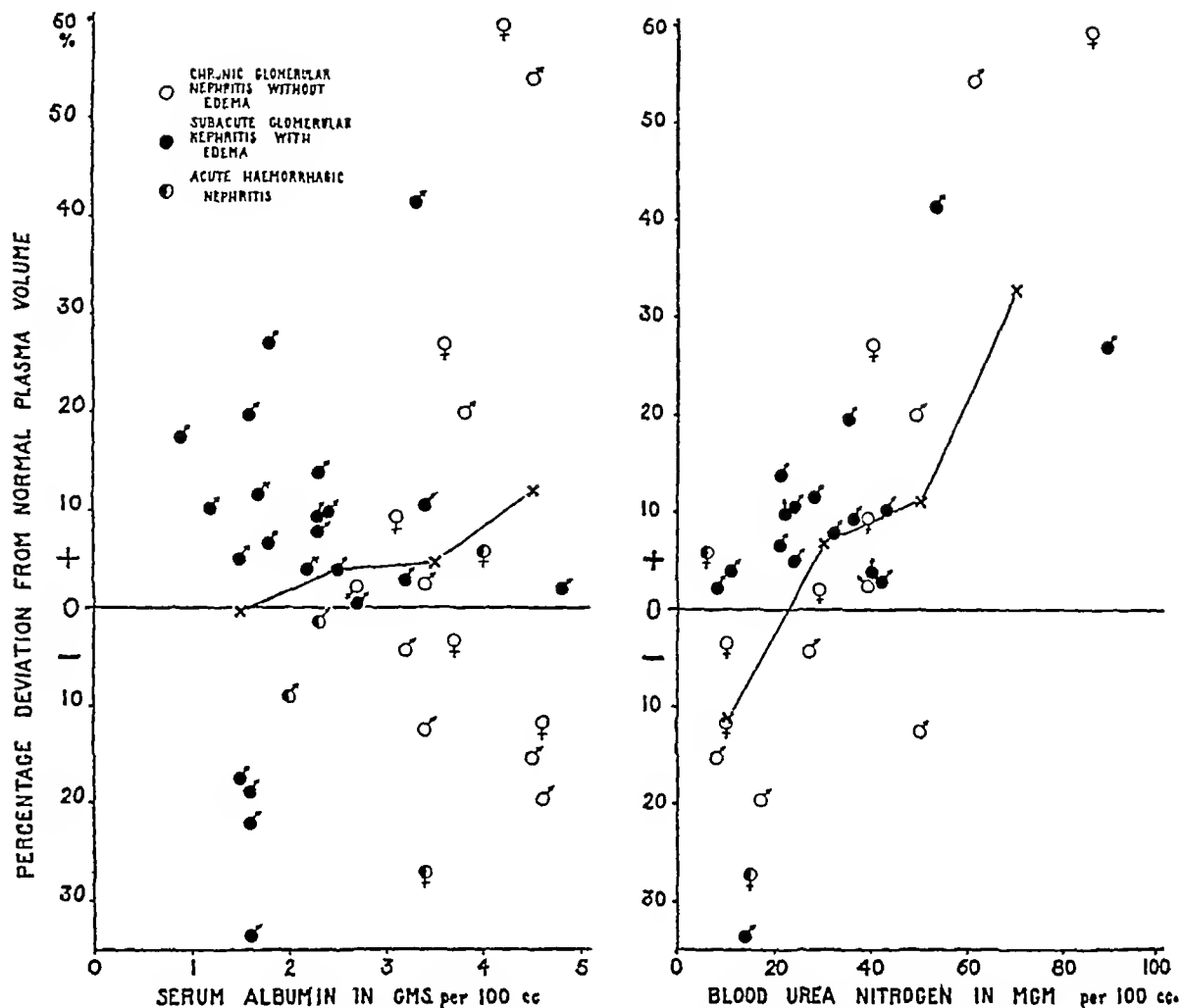


FIG 5 PLASMA VOLUME IN RELATION TO SERUM ALBUMIN AND BLOOD UREA NITROGEN

Increasing concentration of serum albumin and blood urea nitrogen is accompanied by an increase in plasma volume, the average trend of which is indicated by the solid lines

much evidence has accumulated to establish the connection between albuminuria, low plasma protein concentration, decreased osmotic pressure of the plasma, and the development of edema in nephritis (24 to 32). On the basis of present knowledge it is to be expected that the watery element of the blood should be low in hypoproteinemia and that in edematous patients the plasma volume should increase as the serum protein concentration rises under therapy, providing the electrolyte balance remains unaltered. While determinations of blood electrolytes were not made in this study, in our cases of nephritis (see Figure 5) as the nonprotein nitrogen concentration rose, the plasma volume tended to rise. The

mechanism of this augmentation of the plasma volume is not clear.

The work of Blumgart *et al* (34) has shown that in anemia the circulation time becomes faster as the red cell count diminishes. It is interesting that a similar trend was not encountered in the cases of Bright's disease herein studied. The majority of our cases had moderate to severe anemia, and yet the average circulation time as shown in Figure 4 did not definitely become faster as the average red cell count diminished. It is perhaps significant that the circulation time was slower in the cases with subacute glomerular nephritis and with edema (nephrosis syndrome) was, for comparable levels of anemia, slower than the group with chronic

glomerular nephritis. The lowered basal metabolism that obtains in nephrotic cases is well known. The circulation time is slow in myxedema (35-36) even when uncomplicated by anemia. In our opinion the depressed metabolism of the nephrotic cases probably accounts for the relatively slow blood flow at the levels of anemia exhibited by these patients.

There remain for final consideration those cases with renal insufficiency who were in congestive heart failure. In spite of the fact that these patients were moderately dehydrated and cachectic in the later stages of their progressive disease conditions in which one would expect to find a hypovolemia, total blood volume was well above normal limits and considerably higher than the average of either Groups I or II. Circulating red cell volume was above normal in Cases 359, 374, and 393, all of whom had chronic vascular nephritis and had never progressed to the stage of azotemia and anemia which was however the clinical condition preceding congestive failure in Cases 368 and 380. In these latter 2 cases despite their anemia circulating red cell volume was definitely above the average level found at a corresponding red blood cell count in the nephritics without congestive failure. The elevation above normal total blood volume was not as great as the average increase above normal in congestive heart failure due to valvular disease or chronic myocarditis (37).

SUMMARY

In this study we have observed the course of the plasma and total blood volume in nephritis. Three factors appear to be concerned in the regulation of the plasma volume: serum albumin concentration, nonprotein nitrogen concentration, and the degree of anemia. The effect of these factors has been described. Regardless of the stage of the disease, whether acute, subacute, or chronic, with or without edema or renal insufficiency, the level of the plasma volume reflects the interrelationship of these 3 factors. The relationship of the changes in plasma and circulating red cell volume is such that the total blood volume always remains below normal.

With development of congestive heart failure, regardless of the degree of anemia present an

additional factor appears to be introduced resulting in an increased circulating red cell volume and hence increased total blood volume.

CONCLUSIONS

1. In Bright's disease plasma volume tends to vary directly with the serum albumin concentration and blood nonprotein nitrogen concentration and indirectly with the degree of anemia present.

2. In the edematous stage hypoproteinemia tends to diminish and, if present, anemia tends to augment the plasma volume.

3. During diuresis there is a tendency for plasma volume to increase chiefly in relation to the increase in the albumin fraction of the serum protein.

4. The interrelationship of changes in plasma and circulating red cell volume is such that total blood volume is below normal in all stages of the disease.

5. When congestive heart failure occurs in patients with chronic nephritis, plasma circulating red cell, and total blood volume are definitely above average levels found at comparable levels of anemia in patients with chronic nephritis but without congestive heart failure.

6. Circulation time in the group of cases exhibiting subacute glomerular nephritis with edema (nephrosis syndrome) was slower than in the group with chronic nephritis without edema. The lowered metabolism characteristic of the former group may explain this paradoxical finding.

BIBLIOGRAPHY

1. Bright, R., Cases and observations of renal disease accompanied with the secretion of albuminous urine. *Guy's Hospital Reports* 1836 1, 338.
- 2a. Plesch, J., Hamodynamische Studien. *Ztschr f exper Path und Therap.* 1909 6, 459.
- b. Plesch, J., Untersuchungen über die Physiologie und Pathologie der Blutmenge. *Ztschr f klin. Med.* 1922, 93, 260.
3. Schmidt, W., Blutungen Bestimmungen bei Nieren und Herzkrankheiten. *Ztschr f d. ges exper Med.* 1927 58, 276.
4. Waterfield, R. L., Changes in blood volume in patients with edema of renal origin. *J. Clin. Invest.* 1931 9, 589.
5. Linder, G. C., Lundsgaard, C., Van Slyke, D. D., and Stillman, E., Changes in the volume of plasma and absolute amount of plasma proteins in *J. Exper Med.* 1924 39, 771.

- 6 Hartwich, A, and May, G, Blutmengenbestimmungen mittels der Farbstoffmethode. *Ztschr f d. ges exper Med*, 1926, 53, 677
- 7 Litzner, S, Experimentelle und Klinische Untersuchungen über das Verhalten der Blutmenge bei Nierenerkrankungen *Ztschr f Klin. Med*, 1930, 112, 93
- 8 Rusznyak, S, Untersuchungen zur Frage der Gesamtblutmenge des Menschen unter normalen und pathologischen Verhältnissen *Deutsches Arch. f. klin. Med*, 1927, 158, 98
- 9a Brown, G E., and Rowntree, L G, The volume and composition of the blood and the changes incident to diuresis in cases of edema. *Arch. Int. Med.*, 1925, 35, 129
- ✓ 9b Brown, G. E., and Rowntree, L G, Blood volume in edema of glomerular nephritis and nephrosis *Arch Int. Med.*, 1929, 41, 44
- ✓ 9c Brown, G E., Rowntree, L. G., and Roth, G M, The Volume of the Blood and Plasma in Health and Disease. W B Saunders, Philadelphia, 1929
- ✓ 10 Darrow, D C., The blood volume in cases of nephritis with edema and low serum protein concentration *Proc. Soc. Exper Biol and Med*, 1926, 23, 740
- 11 Gibson, J G, 2d, and Evans, Wm. A., Jr, Clinical studies of the blood volume. I Clinical application of a method employing the azo dye "Evans Blue" and the spectrophotometer *J Clin. Invest.*, 1937, 16, 301
- 12 Christian, H A., Clinical varieties of nephritis Interstate Postgraduate Medical Assembly of N A., 1931, 71-74
Evans, W A., Venous pressure. *New England J Med*, 1932, 207, 1934
Winternitz, M, Deutsch, J, and Brüll, Z, Eine Klinisch brauchbare Bestimmungsmethode der Blutumlaufzeit mittels Decholininjektion *Med. Klin.*, 1931, 27, 986
- 13 Osgood, E E, Haskins, H. D., and Trotman, F E., A simplification of the Osgood-Haskins hemoglobin method. *J Lab and Clin. Med.*, 1931, 16, No 5
- 14 Van Slyke, D D, and Cullen, G E, The determination of urea by the urease method. *J Biol Chem.*, 1916, 24, 117
- 15 Folin, O, Laboratory Manual of Biological Chemistry D Appleton-Century Co, New York, 1934
- 16 Koch, F C., and McMeekin, T L., A new direct nesslerization micro-kjeldahl method and a modification of the Nessler-Folin reagent for ammonia. *J Am. Chem. Soc.*, 1924, 46, 2066
- 17 Gibson, J G, 2d, and Evans, W A., Jr, Clinical studies of the blood volume. II Relation of plasma and total blood volume to venous pressure, blood velocity rate, physical measurements, age and sex in ninety normal humans *J Clin. Invest.*, 1937, 16, 317
- 20 Gibson, J G, 2d, Clinical studies of the blood volume. VI Changes in blood volume in pernicious anemia in relation to the hematopoietic response to intramuscular liver therapy *J Clin. Invest.*, 1939, 18, 401
- 21 Gibson, J G, 2d, Harris, A W, and Swigert, V W., Clinical studies of the blood volume. VIII The blood volume in secondary anemia and polycythemia vera. (In preparation.)
- 22 Epstem, A. A., Concerning the causation of edema in chronic parenchymatous nephritis, method for its alleviation *Am. J M Sc.*, 1917, 154, 638
- 23 Starling, E H, The physiological factors involved in the causation of dropsy *Lancet*, 1896, 1, 1407
- 24 Krogh, A., The Anatomy and Physiology of the Capillaries New Haven, 1924
- 25 Moore, N S, and Van Slyke, D D, The relationships between plasma, specific gravity, plasma protein concentration and edema in nephritis *J Clin Invest*, 1929-30, 8, 337
- 26 Barker, M H, and Kirk, E J, Experimental edema (nephrosis) in dogs in relation to edema of renal origin in patients *Arch. Int. Med.*, 1930, 45, 319
- 27 Shelburne, S A, and Egloff, W C, Experimental edema *Arch. Int. Med*, 1931, 48, 51
- 28 Leiter, L, Nephrosis *Medicine*, 1931, 10, 135
- ✓ 29 Chang, H C., Plasma protein and blood volume. *Proc Soc. Exper Biol and Med.*, 1931, 29, 829
- ✓ 30 Lepore, M J, Relation of plasma volume to plasma protein concentration *Proc. Soc. Exper Biol. and Med*, 1932-33, 30, 268
- 31 Krogh, A., Landis, E M, and Turner, A H., The movement of fluid through the human capillary wall in relation to venous pressure and to the colloid osmotic pressure of the blood *J Clin. Invest.*, 1932, 11, 63
- 32 Melnick, D., and Cowgill, G R., The serum protein complex as a factor in regulating blood volume. *Proc. Soc. Exper Biol and Med.*, 1936-37, 35, 312.
- 33 Weech, A. A., The significance of the albumin fraction of serum. *Bull New York Acad of Med.*, 1939, 15, 63
- 34 Blumgart, H L., Gargill, S L, and Gilligan, D R., Studies on velocity of blood flow XV The velocity of blood flow and other aspects of the circulation in patients with "Primary" and secondary anemia and in two patients with polycythemia vera. *J Clin. Invest.*, 1930, 9, 679
- 35 Blumgart, H L., Gargill, S L, and Gilligan, D R., Studies on velocity of blood flow XIV The circulation in myxedema with a comparison of the velocity of blood flow in myxedema and thyrotoxicosis *J Clin Invest*, 1930, 9, 91
- ✓ 36 Gibson, J G, 2d, and Evans, W A., Jr, Clinical studies of the blood volume. III Changes in blood volume, venous pressure and blood velocity rate in chronic congestive heart failure. *J Clin. Invest.*, 1937, 16, 851
- 37 Gibson, J G, 2d, and Harris, A. W., Clinical studies of the blood volume. V Hyperthyroidism and myxedema *J Clin. Invest.*, 1939, 18, 59

INABILITY TO DEMONSTRATE A PLATELET REDUCING SUBSTANCE IN AN ACETONE EXTRACT OF THE SPLEEN FROM PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA¹

By FREDERICK J. POHLE AND OVID O. MEYER

(From the Department of Medicine University of Wisconsin Medical School Madison)

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It is well established that some relationship exists between the spleen and the number of platelets in the circulating blood. Splenectomy performed on patients with chronic idiopathic thrombocytopenic purpura frequently results in a clinical and hematological cure. Recent investigations (1, 2, 3) suggest that the spleen of patients with this disease contains a substance capable of reducing the number of blood platelets in certain laboratory animals. This material obtained by acetone extraction has been called thrombocytopen (3). It was reported that the intravenous injection of thrombocytopen into a normal rabbit reduced the platelet value as much as 90 per cent. Such observations, if confirmed, would be an important contribution to a better understanding of certain purpuric conditions. The purpose of the present investigation was to confirm these recent studies.

METHODS

The material for this study was obtained from the spleens of three patients with typical chronic idiopathic thrombocytopenic purpura. An abstract of the history, physical examination, and laboratory findings in these cases will be given later in this communication.

The method used for the preparation of the acetone extract of each spleen was essentially the same as that described by Troland and Lee (3). In each instance the spleen was taken directly from the operating rooms, finely ground in a food chopper, and placed in five volumes of acetone USP. The flask was kept in the ice box at 5° C. and shaken for 5 minutes daily. After an interval of from 34 to 76 days the light-orange supernatant extract was filtered and the acetone removed from the filtrate by distillation. A yellow

low brown, fatty, sticky residue remained on the walls of the flask. In each case 100 cc. of distilled water was added to the distilling flask, shaken vigorously for 10 minutes, and filtered through one thickness of coarse filter paper. The final preparations were cloudy. Each of the three splenic extracts was used within 48 hours after its preparation.

White male rabbits, approximately 6 months of age, weighing from 2.5 to 4 kilograms, were used as the test animal. A new rabbit was used for every injection. The injections were made into the marginal veins of the ear.

Blood platelet determinations were made with the aid of an isotonic diluting fluid by a method previously described (4). The counts on the purpuric patients were made on capillary blood obtained from the ear; the counts on the rabbits were made on blood obtained from the ear veins. Previous studies have indicated that blood platelet determinations by this method, when performed by an experienced individual, include an approximate technical error of from +4 per cent to -4 per cent.

OBSERVATIONS

Case I. This patient, a white schoolgirl, 16 years of age, was admitted to the hospital with a chief complaint of protracted menorrhagia. The patient had suffered from menorrhagia, bleeding from the gums, frequent epistaxis, and easy bruising for 10 months. During this period 6,500 cc. blood transfusions had been given for anemia. The past history was not pertinent, and there was no family history of any hemorrhagic disorder.

Physical examination upon admission revealed a well developed, well nourished girl with marked pallor. There was bleeding from the gums and vagina. There were numerous petechiae and ecchymotic areas, both old and new.

¹ This study was aided in part by a grant from the Wisconsin Alumni Research Foundation.

entire body The heart and lungs were normal The blood pressure was 136/88 The spleen edge was barely palpable below the left costal margin Aside from the uterine bleeding the pelvic examination was not significant

vealed nothing abnormal The blood Wassermann test was negative X-ray studies of the lungs, paranasal sinuses, and teeth were normal

The patient was observed on the medical service for 6 weeks without change in the clinical or

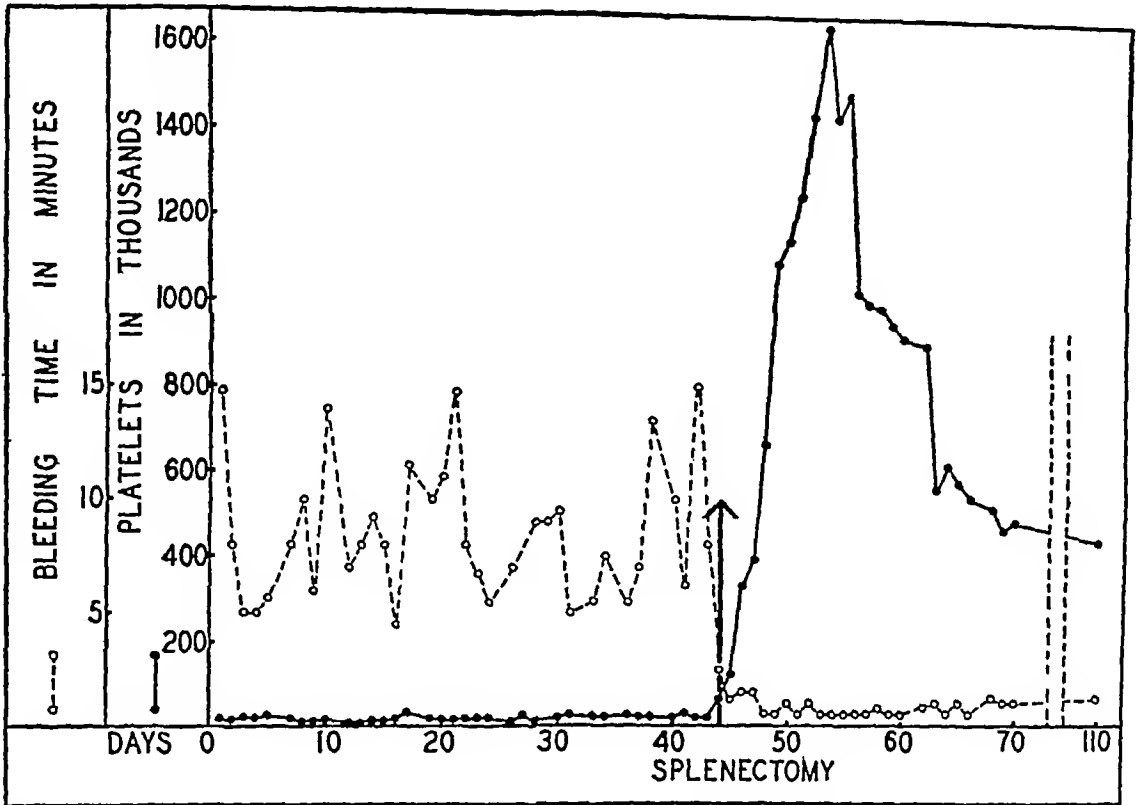


FIG 1 EFFECT OF SPLENECTOMY ON THE BLOOD PLATELET COUNT AND BLEEDING TIME IN AN INDIVIDUAL (CASE I) WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

On admission to the hospital the hemoglobin was 6.36 grams per 100 cc and the erythrocyte count was 2,380,000 per cu mm The leukocyte count was 6,700 per cu mm and the differential count was within normal limits The red blood cells showed moderate variation in size and shape The blood platelet count was 23,000 per cu mm There was no retraction of the clot in 24 hours The bleeding time (Duke's method) was 15 minutes and the coagulation time, determined by placing 2 cc of venous blood in a 100 × 13 mm test tube kept at 37° C, was 8 minutes The tourniquet test was strongly positive The plasma prothrombin (Quick's method) was 100 per cent The blood cevamic acid was 1.31 mgm per 100 cc Urine and stool examinations re-

laboratory status During this time she received 7,500 cc blood transfusions After this period a splenectomy was done The blood platelet count showed a significant increase 4 hours later and all of the hemorrhagic manifestations ceased There has been no recurrence of abnormal bleeding Figure 1 shows the blood platelet counts and bleeding times on this patient during the period of hospitalization The hemoglobin and red blood cells rapidly rose to normal with iron therapy

An extract was prepared from the spleen obtained from this case in the manner previously described The spleen weighed 265 grams An interval of 59 days was allowed for the extraction A portion of this extract (Number 1) was ad-

ministered intravenously to each of three rabbits and the effect on the blood platelet counts was observed. The results are presented in Table I

TABLE I

Effect of intravenous injection of splenic extract Number 1 on the blood platelet counts of three rabbits

Time	Platelets		
	Rabbit number 1 (received 15 cc.)	Rabbit number 2 (received 23 cc.)	Rabbit number 3 (received 50 cc.)
hours	per cu. mm.	per cu. mm.	per cu. mm.
Before injection			
48	415 000	397 000	529 000
24	396 000	420 000	530 000
1	400 000	389 000	558 000
After injection			
1	390 000	367 000	522 000
3	374 000	472 000	
5	387 000	430 000	550 000
7	402 000		546 000
10	425 000	406 000	537 000
20	375 000	359 000	518 000
30	416 000	408 000	493 000
48	387 000	397 000	505 000
72	409 000	414 000	

Case II This patient, a 20-year-old white housewife, was admitted to the hospital because of bleeding from the gums. The patient stated that she had bruised easily for several years. However, during the past four months she had had repeated severe epistaxis bleeding from the gums, menorrhagia and numerous petechiae and ecchymoses over the skin and mucous membranes. Three weeks prior to admission she had had prolonged hemorrhage following two dental extractions. Three blood transfusions were required to keep the hemoglobin from becoming dangerously low. Weakness and ease of fatigue became quite severe. The remainder of the history was irrelevant and there was no hemorrhagic disorder in other members of her family.

On physical examination the patient exhibited marked pallor and lethargy. There was bleeding from the gums and vagina, fresh retinal hemorrhages bilaterally, numerous petechiae over all extremities and in the mouth, splenomegaly and moderate cervical lymph node enlargement. The remainder of the examination revealed nothing abnormal.

On admission to the hospital the hemoglobin was 5.5 grams per 100 cc., and the erythrocytes

numbered 1,920,000 per cu. mm. The leukocyte count was 9,950 per cu. mm. and the differential count was normal. The red blood cells showed microcytosis and a few nucleated forms. Careful examination of the blood smear showed a marked diminution in the number of blood platelets. There was no retraction of the blood clot in 24 hours. The bleeding time was 38 minutes and the blood coagulation time determined by the capillary tube method was 3 minutes. The tourniquet test was positive. The plasma prothrombin (Quick's method) was 100 per cent. The blood civitamic acid was 0.82 mgm per 100 cc. Urine and stool examinations revealed nothing abnormal. The blood Wassermann test was negative. Chest and dental x rays were within normal limits.

There was no essential change in this case during the next three weeks under medical observation. Five 400 cc. blood transfusions were given during this interval without permanent effect upon the bleeding tendency. The method used for the blood platelet counts during this time was not reliable. However the platelets were greatly reduced on repeated examinations of the blood smear and the bleeding time showed values from 10 minutes to 2 hours. A splenectomy was performed and the patient was subsequently observed in the hospital for 10 weeks. Unlike Case I, the hemorrhagic manifestations persisted during this time although they were less severe. The blood platelets never exceeded 35,000 per cu. mm. Seven 400 cc. blood transfusions were given during this postoperative period, and at the time of discharge the hemoglobin was 14.3 grams per 100 cc. and the red blood cell count was 4,540,000 per cu. mm.

This patient has been seen on two occasions in the outpatient department since discharge from the hospital. Four months after splenectomy the blood platelet count was 198,000 per cu. mm. and 7 months after splenectomy the count was 452,000 per cu. mm. The patient has been free of any severe hemorrhages since her discharge from the hospital.

The spleen obtained from this case weighed 310 grams. Immediately after its removal it was ground and placed in five volumes of and an extract was prepared.

instance 76 days were allowed for extraction. A portion of this extract (Number 2) was injected intravenously into each of three rabbits and the effect on the number of blood platelets observed. The data are given in Table II.

TABLE II

Effect of intravenous injection of splenic extract Number 2 on the blood platelet counts of three rabbits

Time	Platelets		
	Rabbit number 4 (received 15 cc.)	Rabbit number 5 (received 25 cc.)	Rabbit number 6 (received 50 cc.)
hours	per cu mm	per cu mm	per cu mm
Before injection			
48		433,000	502,000
24	432,000	502,000	504,000
1	485,000	429,000	557,000
After injection			
1	471,000	486,000	486,000
3	505,000	518,000	554,000
6	437,000	427,000	466,000
10	468,000	478,000	430,000
20	499,000	503,000	558,000
24	538,000		
48	546,000	460,000	501,000
72	462,000	472,000	514,000

Case III This patient, a white boy, age 10 years, was admitted to the hospital with a chief complaint of epistaxis and easy bruising. The patient was in good health until 4 weeks before entry when he first had a severe epistaxis. Purpura became evident over the entire body and on the day previous to admission the patient had 4 emeses of bright red blood. There were no recent infections, and there was no history of an abnormal bleeding tendency in any other member of the family.

Physical examination showed a well developed, well nourished, pale boy with petechiae and purpuric areas over the entire body. The tonsils were hypertrophic and infected. The remainder of the physical examination was essentially normal. The spleen was not palpable.

Laboratory studies on admission showed a hemoglobin of 8.5 grams per 100 cc., an erythrocyte count of 3,300,000 per cu mm., and a leukocyte count of 9,000 per cu mm. The blood smear revealed a normal differential count and changes in the red blood cells consistent with hypochromic anemia. The blood platelet count

was 13,000 per cu mm., and there was no retraction of the blood clot in 24 hours. The bleeding time was 12 minutes, and the venous blood coagulation was 7 minutes. The tourniquet test was positive. The blood cevitic acid was 1.4 mgm per 100 cc. Urine and stool examinations were normal. The blood Wassermann test was negative. X-rays of the lungs, sinuses, teeth, and long bones were normal.

The patient was treated medically in the hospital for 6 months without improvement in the bleeding tendency. The tonsils, and 2 teeth with apical abscesses, were removed with resultant dangerous hemorrhage. During this protracted preoperative period a total of 9,250 cc blood transfusions was given. This patient also received two courses of x-ray therapy to the splenic area. One hundred r (in air) were given over the anterior and posterior spleen on consecutive days up to a total of 400 r over each area. Ten weeks later 200 r (in air) were given over the anterior and posterior spleen on consecutive days up to a total of 800 r over each area. Customary deep x-ray therapy technique was used (half value layer in Cu = 1.0 mm). Repeated blood platelet counts during this time never showed a value of over 58,000 per cu mm., and the bleeding time was persistently prolonged. After this period a splenectomy was performed and the hemorrhages ceased immediately. The response of the blood platelets to splenectomy was very similar to that in Case I as shown in Figure 1. With the cessation of bleeding the anemia rapidly improved. An outpatient visit 3 months after surgery established that there had been no recurrence of abnormal bleeding. The blood platelet count at this time was 244,000 per cu mm.

An acetone extract was prepared from this spleen (weight 70 grams), in the same manner as previously described. In this instance 34 days were allowed for extraction. A portion of this splenic extract (Number 3) was again administered intravenously to each of three rabbits and the effect on blood platelet values noted. The results are shown in Table III.

There were no local or general reactions noted in any of the rabbits as a result of the intravenous injection of the splenic extracts. The animals were carefully watched following the injections but no hemorrhagic manifestations were

TABLE III

Effect of intravenous injection of splenic extract Number 3 on the blood platelet counts of three rabbits

Time	Platelets		
	Rabbit number 7 (received 7 cc.)	Rabbit number 8 (received 24 cc.)	Rabbit number 9 (received 42 cc.)
<i>hours</i>	<i>per cu. mm.</i>	<i>per cu. mm.</i>	<i>per cu. mm.</i>
Before injection			
48	547 000		392 000
24	553 000	415 000	407 000
2	600 000	368 000	385 000
1	565 000	395 000	370 000
After injection			
1	582 000	387 000	339 000
2	599 000	408 000	364 000
4	615 000	404 000	341 000
8	569 000	455 000	359 000
12	578 000	381 000	370 000
20	626 000	400 000	343 000
48	588 000	395 000	351 000

observed. Abnormal bleeding never occurred after the incisions of the ear veins necessary for the numerous blood platelet counts

The gross and microscopic appearances of the spleens were not outstanding. In every case the follicles were quite hyperplastic and the sinusoids

were dilated and filled with phagocytes containing blood pigment. The spleen obtained from Case III showed no fibrosis as a result of the two courses of x ray therapy

CONCLUSION

The present investigations do not confirm previous reports that an acetone extract of the spleen from individuals with chronic idiopathic thrombocytopenic purpura contains a substance capable of reducing the number of blood platelets in rabbits

BIBLIOGRAPHY

1. Torrioli, Mario and Puddu, Vittorio, Recent studies on pathogenesis of Werlhof's disease. *J. A. M. A.*, 1938, 111 1455
2. Troland, Charles E., and Lee, Ferdinand C., A preliminary report on a platelet reducing substance in the spleen of thrombocytopenic purpura. *Bull. Johns Hopkins Hosp.*, 1938, 62, 85
3. Troland, Charles E., and Lee, Ferdinand C., Thrombocytopen substance in extract from spleen of patients with idiopathic thrombocytopenic purpura that reduces number of blood platelets. *J. A. M. A.*, 1938 111 221
4. Pohle, Frederick J., The blood platelet count in relation to the menstrual cycle in normal women. *Am. J. Med. Sc.*, 1939, 197 40

STUDIES IN IRON TRANSPORTATION AND METABOLISM.

III THE NORMAL FLUCTUATIONS OF SERUM AND "EASILY SPLIT-OFF" BLOOD IRON IN INDIVIDUAL SUBJECTS

By CARL V. MOORE, VIRGINIA MINNICH, AND JO WELCH

(From the Department of Medicine Division of Research Medicine and the Ohio Agricultural Experiment Station Division of Home Economics Ohio State University Columbus)

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Recent studies have directed attention to the great theoretical and practical significance of serum or plasma iron. It has been demonstrated, in the first place, that plasma iron is of metabolic importance in that it serves as the medium for iron transportation in the mammalian organism (2, 3, 4, 5). This concept has made possible a more direct approach to the analysis of the factors controlling iron absorption since an index of the amount of iron being absorbed from the intestinal tract under standard conditions can be gained by measuring increases in plasma iron (2, 6, 7). In the second place, the differences which occur in fasting plasma iron values in various of the anemic states have been shown to be of differential diagnostic value (2, 4, 5, 8, 9) and of importance in permitting one to interpret the adequacy of iron reserves, rates of erythrocytogenesis and rates of red blood cell destruction (2, 3).

These several uses to which a study of plasma iron can be applied are dependent upon a thorough knowledge of the fluctuations which may occur in any given individual under normal circumstances. Present information as to the normal range for plasma iron values in the human has been obtained largely from single determinations on a relatively large group of individuals (4, 8, 9 for bibliography prior to 1937, see reference 1). Only a relatively few studies of the variations which occur in the same normal subject from hour to hour or from week to week have been made. Locke, Main and Rosbash (10) noted that the extension of a fasting interval from 12 to 24 hours was attended in normal individuals by a 20 to 40 per cent increase in the serum iron level. Heilmeyer and Plötner (4) obtained variations of plus or minus 30 per cent in the serum iron values of normal persons

when determinations were made at intervals of a few hours or of from 1 to 9 days. Thoenes and Aschaffenburg (11) reported the changes which occurred in serum iron in several healthy infants from the age of 20 days to 8½ months but these figures are of little value for comparison with those from adult human subjects. The same objection applies to those few additional studies which have been made on the daily and weekly variations in the serum iron values of laboratory animals. The present study was undertaken in an attempt to define more sharply the serum or plasma iron fluctuations which occur in hematologically equilibrated subjects and to determine the effect of antianemic therapy on the blood iron relationships of normal individuals.

Interpretation of the metabolic significance of 'easily split off' blood iron has been altered by Barkan and Schales (12) recent observations which tend to show that this form of blood iron is probably dissociated by the action of dilute acids from a pseudo-hemoglobin, an intermediary compound formed during the breakdown of hemoglobin into bile pigment. The previous contention that easily split-off blood iron is directly related to the function of iron transportation has been withdrawn. The normal variations in 'easily split-off' iron values, therefore, are of less importance to a study of this kind, they have been determined however and for the sake of completeness, are included in the presentation.

Methods and spectrophotometric analysis of the intensity of the iron thiocyanate color

Blood specimens were performed in the manner (1). However, since the thiocyanate colorimetric method

challenged on the ground that the color extracted from acid solution by iso-amyl alcohol is not constant, not directly proportional to the concentration of iron present, and is sensitive to minor fluctuations in acidity (13), it was decided to

iron per 20 cc of solution) were prepared which ranged in acidity from 0.5 to 7 normal with respect to sulphuric acid. The ferric state of ionization was assured by the addition of a few drops of concentrated nitric acid to the solutions at

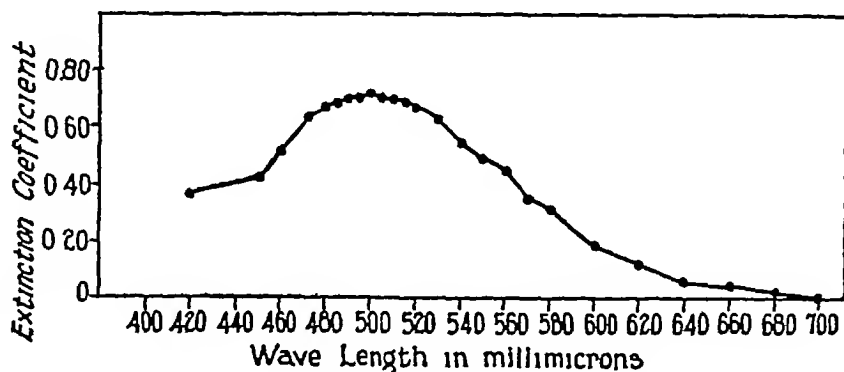


FIG 1 ABSORPTION BAND OF FERRIC THIOCYANATE IN ISO-AMYL ALCOHOL

(0.012 mgm. Fe^{+++} in 10 cc. iso-amyl alcohol, cell depth 25 mm.)

$$\text{Extinction Coefficient} = \log \frac{I_0}{I}$$

Where I_0 is equal to intensity of incident light,

I is equal to intensity of transmitted light.

check the intensity of the color produced under varying conditions with spectrophotometric readings

The absorption band of ferric thiocyanate in iso-amyl alcohol solution is given in Figure 1, it is apparent that maximum intensity of the band was reached at a wavelength of 500 millimicrons. In order to test the effect of changes in acidity on the intensity of color, standard aqueous solutions of ferric iron (0.008 mgm of

approximately 100°C (1). After they had been cooled to room temperature, the solutions were overlaid with 10 cc of iso-amyl alcohol, the color of ferric thiocyanate developed by the addition of 5 cc of a 20 per cent aqueous solution of potassium thiocyanate, and the color immediately extracted by the alcohol. The intensity of the absorption band at a wavelength of 500 millimicrons was determined for all the solutions and found to be remarkably constant (Figure 2)

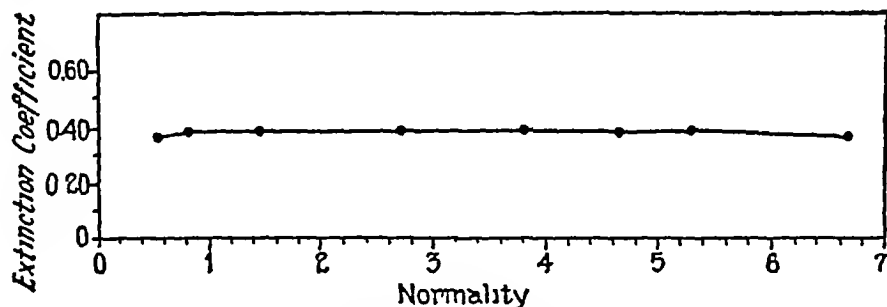


FIG 2. EFFECT OF ACIDITY ON INTENSITY OF THE COLOR OF $\text{Fe}(\text{CNS})_3$ IN ISO-AMYL ALCOHOL—SPECTROPHOTOMETRIC COMPARISONS

Normality refers to the iron solution from which the $\text{Fe}(\text{CNS})_3$ was extracted by iso-amyl alcohol, 20 cc. iron solution (0.008 mgm. Fe) and 10 cc. iso-amyl alcohol were used. Normality variation was effected by changing the amount of sulphuric acid. Readings were made at a cell depth of 20 mm., wavelength 500 millimicrons

Furthermore, the point of maximum intensity of the band did not shift appreciably from the 500 millimicron wavelength mark, a fact which indicates that the quality of the thiocyanate color remained approximately unchanged. The acidity variations attained here far exceed the 2 to 4 normality range actually encountered in serum iron determinations as done in these investigations (1). Further weight is thus added to the contention that no more accurate control of

from acid solution (normality limits as above described) by iso-amyl alcohol was directly proportional to the concentration of iron. Again, the maximum intensity of the absorption band remained relatively constant at 500 millimicrons wavelength.

It was further ascertained that the ferric thiocyanate color in iso-amyl alcohol solution remained practically constant in a darkened room for at least one hour. If the solutions were ex-

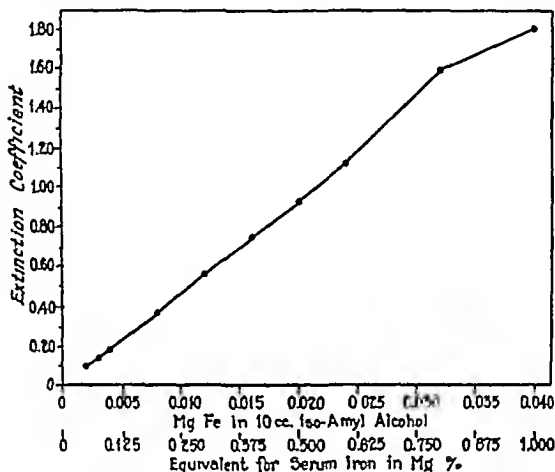


FIG. 3 SPECTROPHOTOMETRIC COMPARISON OF THE INTENSITY OF THE $\text{Fe}(\text{CNS})_3$ COLOR IN ISO-AMYL ALCOHOL PRODUCED BY DIFFERENT AMOUNTS OF Fe^{++}

Readings made at cell depth 20 mm., wavelength 500 millimicrons.

acidity than this is necessary to insure the accuracy of the method.

Solutions of iron were then prepared to correspond to serum iron variations from 0.05 to 1.0 mgm. per cent (0.002 to 0.04 mgm. of iron in 20 cc. of solution to be extracted by the 10 cc. of iso-amyl alcohol). The extinction coefficients of these specimens were measured at a wavelength of 500 millimicrons and plotted on a graph (Figure 3). Within the limits of error of the visual readings the curve described by the intensity values of the color was a straight line, indicating that for the concentrations of iron used, the color of ferric thiocyanate extracted

posed to direct sunlight or to strong artificial light, however, considerable fading occurred during a 60-minute period.

The authors wish to confirm the observation of Barkan, Heilmeyer, Baer, and others that if serum is diluted with one or two volumes of 0.1 N HCL and permitted to stand at room temperature for 15 to 30 minutes before trichloroacetic acid is added, the iron is ionized or so changed that it passes quantitatively into the filtrate and is not brought down with the protein trichloroacetic acid precipitate. The use of this method of precipitation frees the serum of any hemo-

difficult benzidine method of measuring traces of hemoglobin

Total red blood cell counts were made on blood obtained by finger puncture. The counting chambers and Trenner diluting pipettes used were certified by the U S Bureau of Standards. Hemoglobin determinations were done by the acid hematin method with a Dubosq colorimeter and the Newcomer standard color disc. Wintrobe tubes were used for the estimation of packed cell volume, the anticoagulant used was a mixture of

a 24-hour period on a number of normal subjects and hospital patients. The fluctuations were relatively slight, 20 to 35 micrograms per 100 cc in serum iron values and 15 per cent in the "easily split-off" iron levels. On one of the subjects, V M, a young woman aged 28 years, 2 sets of observations at intervals of 1 week were obtained (Figure 4). During the first period, a change of 35 micrograms per cent in serum iron was observed, but during the second period, the amount remained practically constant. Figure 5

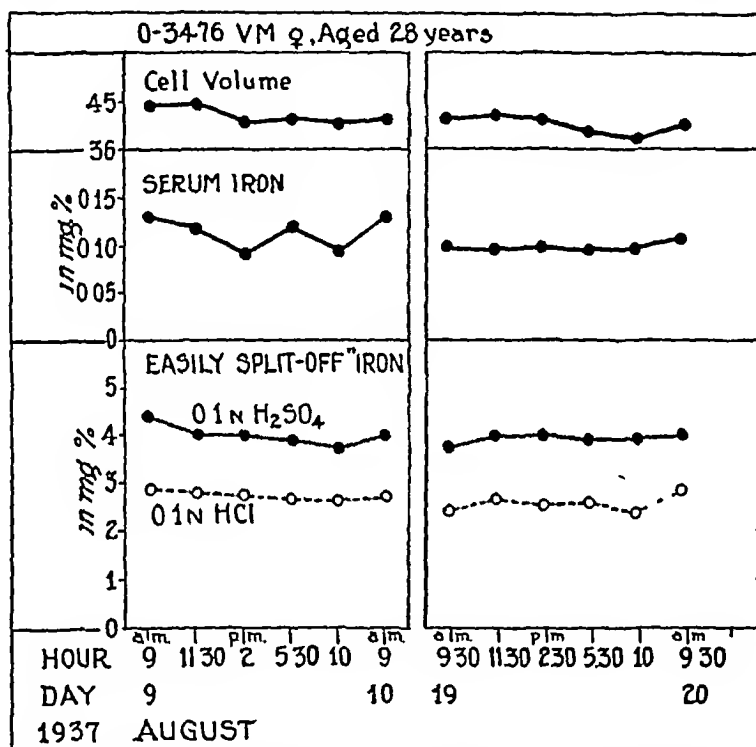


FIG 4 VARIATIONS IN SERUM AND "EASILY SPLIT-OFF" IRON IN A NORMAL SUBJECT DURING TWO 24-HOUR PERIODS

ammonium oxalate and potassium oxalate, as recommended by Heller and Paul (14), the specimens were centrifuged at 3,000 r p m for 30 minutes

Intra diem variations in serum and "easily split-off" blood iron

In order to study the quantitative variations which occur in the various blood iron fractions during the daily cycle, we made determinations on 6 or 7 blood samples taken at intervals throughout

records the fluctuation of serum and "easily split-off" blood iron during 24 hours in a white male, aged 55 years, who was a patient in the University Hospital with a diagnosis of chronic alcoholism, portal cirrhosis, and a functional achlorhydria. The values were remarkably constant. It should be noted that the serum iron level in this patient was below the zonal range usually found in adult males, and that a moderate anemia was present. The changes observed in all subjects were not constant in direction, but oscillated and were independent of normal food intake.

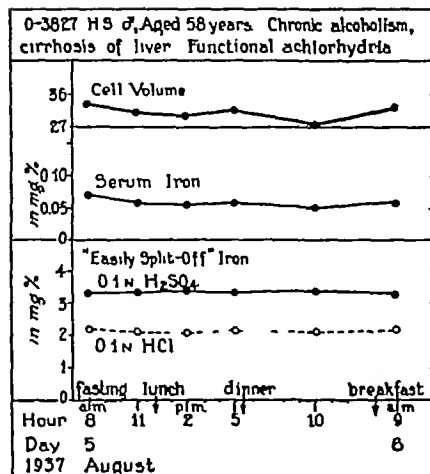


FIG. 5 VARIATIONS IN SERUM AND "EASILY SPLIT OFF" IRON DURING A 24-HOUR PERIOD IN A PATIENT WITH FUNCTIONAL ACHLORHYDRIA

Variations in the blood iron fractions of normal female subjects during 6 months of observation

During the time this study was being made, 16 "normal" Freshman college women were subjecting themselves as a part of another project, to complete finger blood counts biweekly. These young women permitted venipunctures to be done at intervals of 4 weeks for 6 months in order that serum and "easily split-off" iron determinations might likewise be obtained. They reported to the laboratory between 8 and 9 o'clock without breakfast on the same morning of each week that blood was to be drawn. Minimum and maximum values for the various data accumulated are summarized in Table I. The variations of approximately one million in total red cells were no greater than those observed by Doan and Zerfas (15) when consecutive counts at intervals of 15 minutes over a period of hours were obtained on normal subjects. Hemoglobin and packed cell volume changes closely paralleled those for the red cells.

The lowest serum iron value observed during

TABLE I

Extremes of variation in serum and "easily split-off" iron observed during six months of study in sixteen normal college women

Case	Hematological data						Iron studies						Menstrual flow			
	Num-ber of deter-minations	Red blood cells		Hemoglobin		Cell volume		Num-ber of deter-minations	Serum iron		"Easily split-off" iron				Month	Amount of blood
		Mini-mum	Maxi-mum	Mini-mum	Maxi-mum	Mini-mum	Maxi-mum		HCl		H ₂ SO ₄					
									Mini-mum	Maxi-mum	Mini-mum	Maxi-mum				
		millions		grams per cent		per cent			mgm per cent		mgm per cent		mgm per cent			cc.
G D	11	4.28	5.18	13.4	15.5	41	45	5	0.102	0.156	3.00	3.43	4.09	5.09	May	15
F F	11	4.07	4.89	12.6	15.2	35	44	6	0.105	0.140	2.54	3.66	3.95	4.76	April	25.2
H G	13	4.15	4.86	12.1	14.2	41	44	5	0.080	0.135	2.61	3.17	3.32	4.80	May	16
B H	10	4.03	4.82	13.1	14.9	41	43	5	0.096	0.127	2.83	3.14	4.00	4.95	May	9
I L	11	3.56	4.59	12.1	15.3	38	42	5	0.139	0.178	2.51	3.97	3.82	4.31	April	35
E L	12	3.43	4.33	11.3	12.8	36	40	6	0.068	0.115	2.46	3.24	3.64	4.41	May	22
D M	12	3.80	4.91	11.6	14.2	37	42	5	0.082	0.120	2.23	2.83	3.82	4.58	April	12
D M McC	11	3.88	4.96	12.6	15.2	39	45	6	0.119	0.172	2.41	3.33	3.77	4.88	May	29
E M	12	3.50	4.51	11.8	13.2	38	42	6	0.093	0.157	1.85	2.86	3.46	4.36	April	23
G P	7	3.81	4.43	11.3	14.1	39	43	3	0.102	0.156	2.71	3.12	4.02	4.29		
A R.	8	3.90	5.19	12.0	14.3	39	45	5	0.116	0.158	2.73	3.49	4.00	4.32	April	25
J S	11	3.93	4.54	10.9	12.8	36	39	5	0.086	0.113	2.35	3.14	3.55	4.02		
M T	12	3.79	4.60	11.3	14.9	41	43	5	0.096	0.141	2.26	3.73	3.97	4.65	May	20
V W	10	3.46	4.68	11.3	14.2	38	42	6	0.076	0.117	2.69	2.91	3.54	4.58	May	25.1
N W	10	3.86	4.76	11.8	14.1	40	44	5	0.122	0.146	2.47	3.09	4.00	4.61	April	41
L McH	8	3.71	4.41	10.9	13.6	41	44	3	0.113	0.131	2.91	3.28	4.03	4.41	May	12
Average difference									0.041		0.77		0.78			

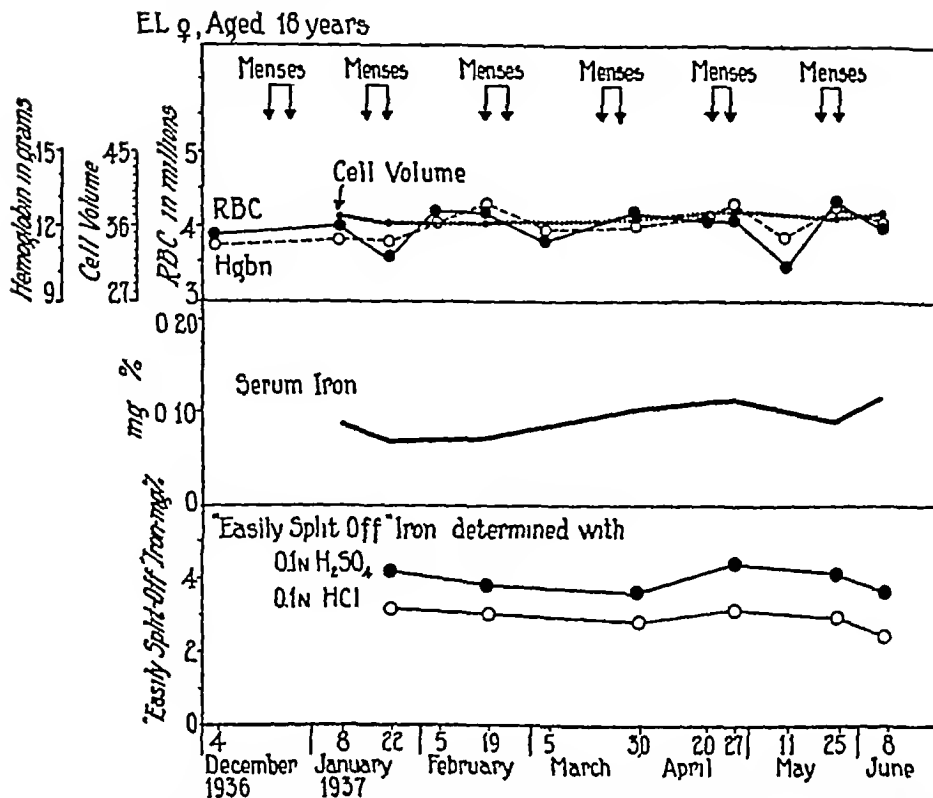


FIG. 6 RELATIVE CONSTANCY OF SERUM AND "EASILY SPLIT-OFF" IRON IN A NORMAL SUBJECT DURING FIVE MONTHS OF OBSERVATION

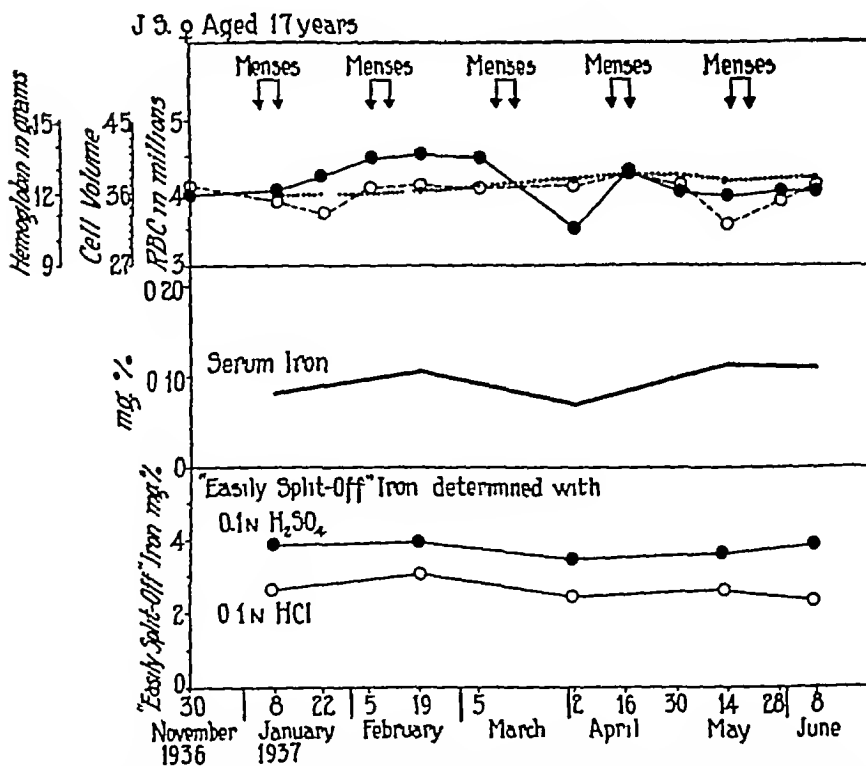


FIG. 7 RELATIVE CONSTANCY OF SERUM AND "EASILY SPLIT-OFF" IRON IN A NORMAL SUBJECT DURING FIVE MONTHS OF OBSERVATION

pads were extracted repeatedly with additional amounts of 0.1 N HCl. Seven to 10 liters of the dilute acid were usually used for one set of menstrual pads. An aliquot portion of the extract was taken for digestion and the determination of iron. A number of unused pads was extracted in a similar manner in order that correction might be made for the iron present in them. For the purpose of calculating the maximum menstrual

Effect of specific antianemic therapy on the blood iron fractions of hematologically equilibrated individuals

It is of theoretical interest to know whether it is possible to increase the serum iron level of normal individuals by giving them iron in therapeutic amounts so as to saturate their storage depots. Accordingly, various iron salts—ferrous sulfate,

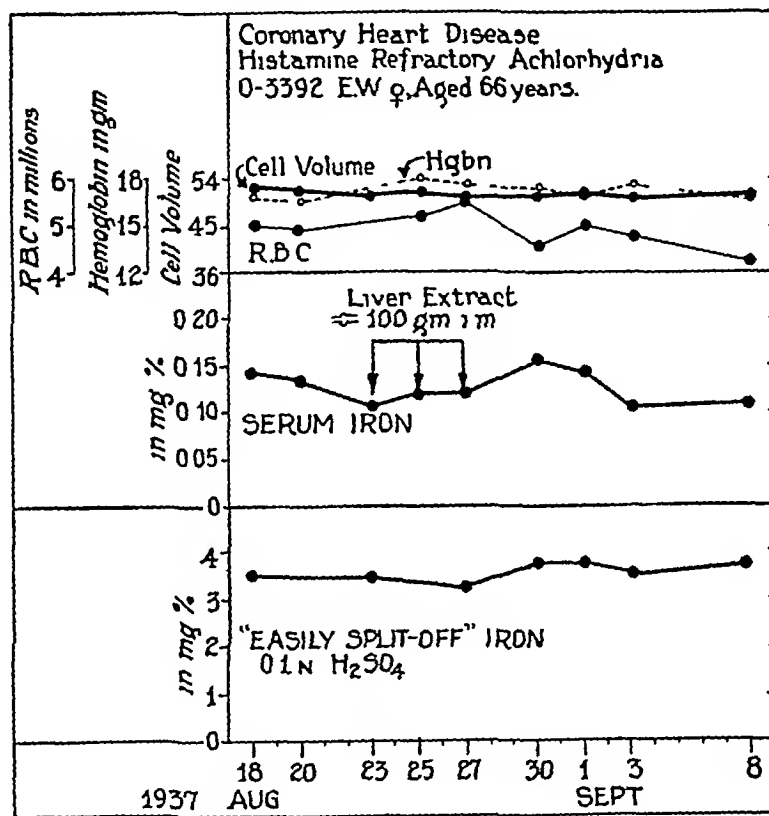


FIG 9 EFFECT OF PARENTERALLY ADMINISTERED LIVER EXTRACT ON BLOOD IRON RELATIONSHIPS IN A SUBJECT WITH NORMAL BLOOD PICTURE

flow, it was assumed that all the iron had been discharged as hemoglobin. Even with this assumption, the loss in terms of volume of blood (calculated on the basis of the subject's hemoglobin level) ranged only from 9 to 41 cc. It was not possible to correlate the serum iron or hemoglobin levels with the quantity of menstrual flow, that is, girls with the largest menstrual loss did not necessarily have the lowest serum iron and hemoglobin values.

ferrous carbonate, iron pyrophosphate, ferric sodium citrate, ferric ammonium citrate—were taken by a number of healthy adult subjects in ordinary therapeutic doses for periods of time which varied from 4 to 8 weeks. In no instance was any significant change in either serum or "easily split-off" blood iron values noted. The data from one of these observations are graphically recorded in Figure 8.

Parenteral injection of liver extract likewise

failed to influence the level of the blood iron fractions in subjects with normal blood pictures. One of the patients on whom these negative results were obtained is of interest in that he had a histamine refractory achlorhydria (Figure 9).

DISCUSSION

When one considers that serum or transport iron must at all times be in physiological equilibrium with the amount of the metal being absorbed from the gastro-intestinal tract, being utilized by the bone marrow for hemoglobin synthesis, being excreted, being added to or withdrawn from the storage depots, and that being thrown into the blood stream as the hemoglobin from destroyed red cells is broken down to bile pigment, then the fluctuations of 10 to 65 micrograms per cent observed in the normal subjects studied in this investigation are not surprising. These changes within the normal zonal range were oscillating in nature and had none of the specific directional characteristics which are so typical of those changes which invariably have been found to result from disturbances of the erythrocytogenic equilibrium (2, 3).

The failure of liver extract to influence serum iron levels of normal individuals was an expected finding but the inability of orally administered iron to raise the fraction above normal levels is particularly interesting. The experiments of Fowler and Barer (16) and of Brock and Hunter (17) indicate that amounts of iron as great as 6 to 88 grams may be retained by the human subject during one month of ordinary therapeutic administration amounts which are over twice the quantity of iron present in the circulating blood of an average adult male. If the equilibrium between serum and stored iron is purely physicochemical in nature, it would be reasonable to expect a decided increase in serum iron as the storage depots become more adequately supplied. Since such an increase did not occur during periods of 6 to 8 weeks of iron therapy, it is suggested that either (1) a regulating mechanism operative under normal conditions, controls the serum iron level or (2) a portion of absorbed iron is deposited in tissues possibly in skin, in a form which removes it temporarily at least from availability to the body economy.

As has already been stated 'easily split-off' iron is of less theoretical interest in any consideration of problems related to iron transportation because evidence has been presented which tends to show that it is derived from a compound formed as an intermediary stage in the breakdown of hemoglobin to bile pigment (12).

CONCLUSIONS

1 The color of ferric thiocyanate in 180-amyl alcohol, extracted from an acid (H_2SO_4) aqueous solution was found to be

- (a) Practically constant for a period of an hour, when kept in a darkened room
- (b) Unchanged both in intensity and quality when the acid solution from which it was extracted varied in acidity from 2 to 4 normal
- (c) Directly proportional in intensity to the amount of iron present, at least within the range of 0.002 to 0.04 mgm of iron per 10 cc. 180 amyl alcohol. This amount of iron corresponds with the method used, to a variation of serum iron from 0.05 to 1.0 mgm per cent.

2 Differences between minimum and maximum serum iron values in a given normal subject ranged within the daily cycle and during a 6-month period from 0.01 to 0.065 mgm per cent. These changes were oscillating and distinctly not specifically directional in character.

3 Liver and iron medication to the normal subject did not effect any appreciable alteration in the serum iron level.

4 'Easily split-off' iron varied in the normal human adult as much as 148 mgm per cent. This fluctuation was likewise oscillating in type and unrelated to liver and iron therapy when the latter was given. The physiological importance of this blood iron fraction has fairly definitely been divorced from the function of iron transportation.

BIBLIOGRAPHY

- 1 Moore, Carl V., Studies in iron transportation and metabolism, chemical methods and normal values for plasma iron and "easily split-off" blood iron. *J. Clin. Invest.*, 1937, 16, 613.

- 2 Moore, Carl V, and Doan, Chas A, Mechanism of iron transportation its significance in iron utilization in anemic states of varied etiology J Clin. Invest. (Proc.), 1936, 15, 455
- Moore, Carl V, Doan, Chas A, and Arrowsmith, Wm. R, Studies in iron transportation and metabolism II The mechanism of iron transportation its significance in iron utilization in anemic states of varied etiology J Clin Invest., 1937, 16, 627
- 3 Moore, Carl V, and Doan, Chas, A, Correlation of serum iron, bone marrow and blood cell changes following specific therapy in the macrocytic anemias Arch Path. (Proc.), 1937, 23, 738
- 4 Heilmeyer, Ludwig, and Plötner, Kurt, Das Serum-eisen und die Eisenmangelkrankheit. (Pathogenese, symptomologie und therapie.) Jena, Gustav Fischer, 1937
- 5 Baer, P, Il ricambio del siero in diverse condizioni in rapporto al ricambio emoglobinico, Kongressbericht II des XVI Internationalen Physiologen-Kongresses, 1938, p 344
- 6 Moore, Carl V., Arrowsmith, Wm. R., Welch, Jo, and Minnich, Virginia, Studies in iron transportation and metabolism. IV Observations on the absorption of iron from the gastro-intestinal tract. J Clin. Invest, 1939, 18, 553
- 7 Hahn, P F, Bale, W F, Lawrence, E. O, and Whipple, G H, Radioactive iron and its metabolism in anemia. J A. M A., 1938, 111, 2285
- 8 Van Goidsenhoven, F, Hoet, J, and Lederer, J, Le fer Serique en clinique humaine. Revue belge Sc. méd., 1938, 10, 177
- 9 Walker, B S, The iron of human blood serum. J Lab and Clin Med, 1938, 24, 308
- 10 Locke, A, Main, E. R., and Rosbash, D O, Copper and non-hemoglobinous iron contents of blood serum in disease. J Clin. Invest., 1932, 11, 527
- 11 Thoenes, Fritz, and Aschaffenburg, R, Der Eisenstoffwechsel des Wachsenden Organismus Abh aus der Kinderh u ihren Grenzgebieten, Verlag von S Karger, Berlin, 1934, 35
- 12 Barkan, G, and Schales, O, Chemischer Aufbau und Physiologische Bedeutung des "Leicht Ab-spaltbaren" Bluteisens 13 Mitteilung in der Reihe der Eisenstudien. Ztschr f Physiol Chem., 1937, 248, 96
- 13 Jenkins, C E., and Thomson, M L, Distribution of iron in blood Brit. J Exp Path., 1937, 18, 175
- 14 Heller, V G, and Paul, H, Changes in cell volume produced by varying concentrations of different anticoagulants J Lab and Clin. Med, 1934, 19, 777
- 15 Doan, C A, and Zerfas, L. G, Rhythmic range of white blood cells in human, pathological leucopenic and leucocytic states, with study of thirty-two human bone marrows J Exp Med., 1927, 46, 511
- 16 Fowler, W M, and Barer, A P, Retention and utilization of orally administered iron. Arch. Int. Med, 1937, 59, 561
- 17 Brock, J F, and Hunter, D, Fate of large doses of iron administered by mouth Quat. J Med., 1937, 6, 5

STUDIES IN IRON TRANSPORTATION AND METABOLISM

IV OBSERVATIONS ON THE ABSORPTION OF IRON FROM THE GASTRO-INTESTINAL TRACT^{1, 2}

By CARL V. MOORE, WM. R. ARROWSMITH,³ JO WELCH AND VIRGINIA MINNICH

(From the Department of Medicine, Division of Research Medicine, Ohio State University, Columbus)

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Most of the problems of iron therapy which have troubled physicians during the past half century are fundamentally traceable to our ignorance of the mechanism by which iron is absorbed from the intestinal tract. Had that mechanism been understood, the discussions centering about the relative efficacy of inorganic and organic iron preparations would not have occurred and hematologists would not now be troubled by the clinical observation that simple ferrous salts are apparently utilized more completely for hemoglobin formation than are the complex ferric salts, such as iron and ammonium citrate (5 to 11 mc.) Adequate experimental analysis of the factors involved in iron absorption has been delayed by the lack of a method sufficiently dependable to measure immediate absorption from the intestinal tract. Investigators have been forced to utilize indirect procedures which measure (1) the difference, in a given period of study between the amount of iron ingested and the amount excreted in the urine and feces (12, 13), (2) the amount of iron which "disappears" from an isolated segment of intestine (14) or (3) the total increase in circulating hemoglobin resulting from the therapeutic administration of iron in various forms. From the hemoglobin increase is then calculated the percentage of iron that has been utilized (15, 16, 17). The first of these three approaches is open to the objection that no account can be taken of

the iron which may be absorbed and then excreted into the colon (18). McCance and Widdowson have however, presented evidence which tends to show that such excretion is minimal (19). The technical difficulties are likewise significant in that the quantities of ingested and excreted (fecal) iron are large whereas the amount actually retained by the body has been shown to be comparatively small. Under such circumstances, the probable error in the determination of the large amounts administered orally and excreted in the feces is great and the period of study must at least be an extended one if the differences obtained are to be regarded as significant. The second approach is that in which, by a process of intubation a known amount of iron is placed in an isolated segment of intestine. After a period of time, the contents of the segment are aspirated and the difference between the quantity of iron originally added and that recovered is assumed to be the amount that has been absorbed. Groen and Taylor (20) have demonstrated, however, that the difference is largely due to iron retained because of adsorption by the mucosa, rather than to actual absorption. While the third method has certain advantages it not only is time consuming and indirect but has recently been challenged on the ground that much more iron is actually absorbed and retained by the body following therapeutic than is utilized for immediate hemoglobin synthesis (12, 21, 22, 23).

If however, iron absorption could be studied directly and immediately by measuring the amount added to the blood stream by the resorptive process many of the difficulties would be eliminated. This approach became possible only after it had been demonstrated that iron is transported as plasma or serum iron (2, 4). Its use is dependent upon a knowledge of the

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³ M & R Dietetic Laboratory Fellow in Medical Research, 1937-1938.

relationships of iron in the blood. Iron occurs in the blood stream in three states as hemoglobin iron, as "easily split-off" iron, and as plasma iron. The first of these is by far the largest in amount and both its chemical nature and physiological function are inseparably linked with that of the hemoglobin molecule. "Easily split-off" iron constitutes 5 to 10 per cent of the total blood iron, its physiological function and chemical nature have not definitely been defined, but Barkan and Schales (24) have presented evidence to suggest that it is derived from a "pseudo-hemoglobin," possibly an intermediate step in the breakdown of hemoglobin into bile pigment. Cortis-Jones and Lemberg are apparently in agreement with this point of view (25). It is entirely possible, as these authors suggest, that other iron containing pigments, degradation products of hemoglobin, are likewise present in the peripheral blood, but since these substances are closely related, physiologically at least, to Barkan's "pseudo-hemoglobin," they may be considered together with the latter as forming one type of blood iron compound. Serum or plasma iron is present in the relatively minute quantities of 50 to 180 micrograms per cent, its chemical nature is in question, but it

probably is part of a complex radical, in the ferric of ionization, in combination with serum globulin (1), the function of serum iron, as has been stated, is apparently that of iron transportation.

Demonstration has already been made of the facts that (1) under basal conditions of iron intake and utilization, hourly variations in the serum iron level are comparatively slight (3), and (2) following the oral administration of a single large dose of various of the iron salts, there is a prompt rise in the serum iron fraction (2). This increase is apparent within the first half hour, reaches its maximum in $2\frac{1}{2}$ to 5 hours, and then gradually falls to approximate the basal level by the end of 12 hours. The serum iron increase is not associated with a rise in the serum bilirubin content. Hemoglobin and "easily split-off" iron fractions do not participate in the change (Figure 1).⁴ By following the serum

iron responses to graded amounts of orally administered iron salts, therefore, under contrasting states of gastric acidity, during varying states of hematopoietic activity, and with experimentally altered conditions of intestinal motility and absorption, it should be possible theoretically to obtain considerable information about those factors which influence and control iron absorption. The present communication presents the results of such a study.

Statement has previously been made (2) of the fact that other workers have noted the increase in serum iron which occurs when iron salts are taken by mouth. Thoenes and Aschaffenburg (27) and Heilmeyer and Plötner (28) made, in addition, a comparison of the rise in serum iron produced by several of the iron salts under the influence of various conditions which might alter intestinal absorption. The results of these studies will be discussed in connection with the analysis of the data accumulated in this investigation. These authors were not primarily interested in the problem of iron absorption and did not, therefore, fully apply the serum iron absorption curve technique to the study of the subject.

It must be emphasized that, since serum (transport) iron is influenced not only by the iron being absorbed from the intestinal tract, but also by that being withdrawn from, or added to, the blood stream by the organs of storage, utilization, blood cell destruction, and excretion, the variations in serum iron do not measure the actual amount of iron being absorbed. The curve of serum iron does, however, indicate the fact and the degree of absorption. This method of study, furthermore, has a decided advantage in that it permits comparison of responses in the same individual not only to different iron preparations, but also to the same salt at different periods of hematopoietic activity, and under different gastro-intestinal influences.

cells. The following statement is made (26, p 2286): "The rapid appearance of radioactive iron in the red blood cells is of great interest. A discussion of these observations must await further experiments to indicate the relationship of this iron to the hemoglobin of the various types of red blood cells (nucleated, immature, and mature)." In normal dogs the iron did not appear to be absorbed in comparable amounts.

⁴Hahn, Bale, Lawrence, and Whipple (26) fed anemic dogs either ferric chloride or ferric sulphate which contained radioactive iron and noticed a prompt increase in radioactive iron, first in the plasma, and later in the red

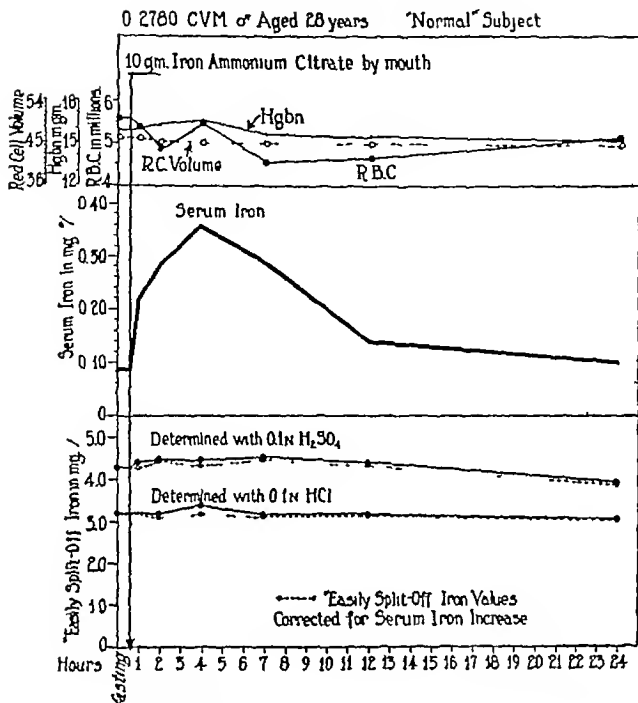


FIG. 1 ABSORPTION OF IRON FROM INTESTINAL TRACT REFLECTED BY AN INCREASE IN SERUM IRON

No change occurs in the "easily split-off" iron fraction.

The collection of serum specimens and the technic of quantitative iron analyses were carried out in the manner previously described (13)

The rôle of the lymph in iron absorption

Since Gaule's (29) claim that when ferric chloride was given orally to rabbits he was able to demonstrate iron by staining methods in the thoracic duct lymph there has been considerable question as to the function of the intestinal lymph passages in iron absorption. Gaule's experiments were criticized by Müller (30) on the ground that ferric chloride is a protein precipitant and could easily cause considerable damage to the readily injured mucous membrane of the rabbit's intestinal tract. Müller repeated the experiment

using ferri-oxytartaricum and failed to demonstrate any increase of iron in the lymph of the thoracic duct. These experiments were performed before chemical methods of sufficient sensitivity to permit chemical quantitation of the iron had been developed. Further credence was given to the possibility of lymph transportation by the histological experiments of Höber (31), which tended to indicate that iron is resorbed intra-epithelially along with lipid substances whereas lipid insoluble material seemed to be absorbed inter-epithelially. This conception of iron absorption as a function of living epithelial cells is compatible with Lintzel's (32) Fontes and Thivolle's (33) and Hahn's *et al.* (26) belief that the body is capable of selective absorption iron

—that is, of assimilating or rejecting it according to the needs at any given time.

In order to study this problem further, both the blood serum and the thoracic duct lymph iron were followed in a number of dogs after the giving of moderately large doses of iron by stomach tube. The dogs were prepared for experiment by being fasted for 18 hours. They were then fed a meal which consisted of one-half pound of lard and a small amount of ground beef muscle. Four to 5 hours later, the animals were anesthetized by intra-peritoneal injection of nembutal. The thoracic duct was isolated and cannulated. "Fasting" lymph and serum specimens were obtained and the iron salt, dissolved in 100 cc of water, was permitted to run into the stomach through an ordinary lavage tube. All of the lymph was collected into 50 cc paraffined centrifuge tubes, the samples were pooled for each successive 30- or 60-minute period and centrifugalized to rid them of any red cells present. Serum specimens were collected at hourly intervals from blood obtained by femoral arterial puncture. Since observations were continued for 6 to 10 hours or more, and since considerable fluid was withdrawn from each animal, as blood and lymph, subcutaneous injections of 100 cc saline were made throughout the course of the experiment at intervals of $1\frac{1}{2}$ to 2 hours. Control

hematocrit determinations, total cell counts, and hemoglobin estimations were likewise made. That the iron level in both serum and lymph was relatively constant under the conditions of "iron-fasting" obtained in these animals with an undisturbed hematopoietic equilibrium, is indicated in the representative data presented in Figure 2. These results of 1 experiment in which 2 grams of ferrous sulphate were given are tabulated in Figure 3. The serum iron slowly increased to approximately twice its basal level, remained at that comparatively high figure for several hours, and then began to fall slightly by the time the experiment was terminated. The rise of iron in the lymph, however, was not nearly as great, and occurred much more slowly. It is interesting to note that the total amount of lymph collected in the 6 hours of observation was only 161 cc and that the total increase of iron in the whole of that volume was not as great as the increase in 100 cc of serum at the height of the serum iron absorption curve.

The delayed increase in lymph iron is not difficult to explain since lymph drains the intercellular fluid and the latter is supplied, in part at least, from the blood stream. It is natural, then, to expect that a rise in serum iron would be reflected by a rise of that metal in the lymph. Experimental confirmation of that postulate was ob-

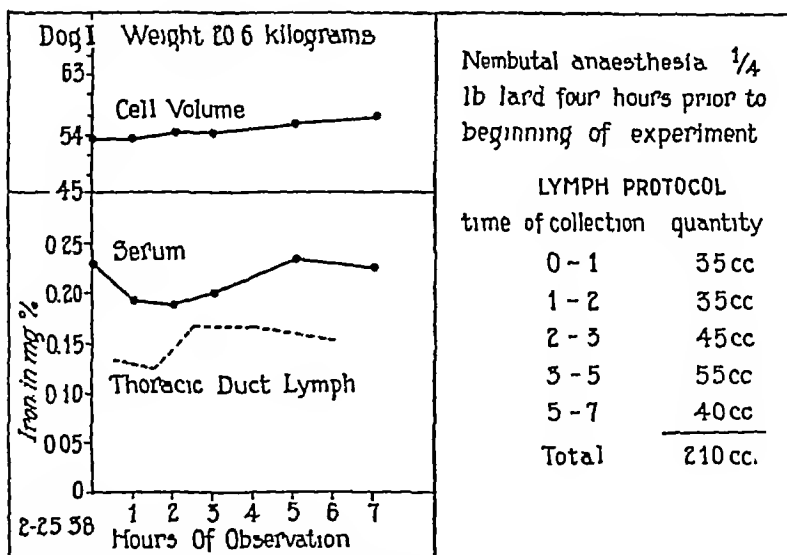


FIG. 2. RELATIVE CONSTANCY OF SERUM AND THORACIC DUCT LYMPH IRON UNDER "IRON-FASTING" CONDITIONS IN THE DOG

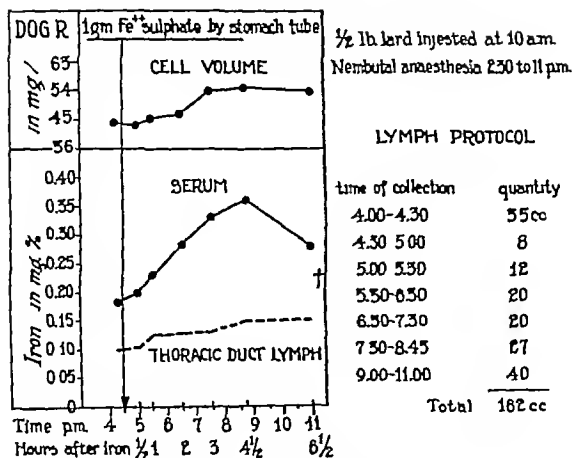


FIG. 3 IRON IN BLOOD SERUM AND THORACIC DUCT LYMPH FOLLOWING ORAL ADMINISTRATION OF SINGLE DOSE OF FERROUS SULPHATE

tained by injecting iron intravenously into dogs after the thoracic duct had been cannulated. Under these conditions the serum iron was increased immediately to high levels (1 mgm per 100 cc. or more) and fell slowly during the subsequent 12 hours to approximate the pre injection level. Iron in the lymph was increased within half an hour, reached its maximum rise at the end of 2 or 3 hours and then slowly began to fall (Figure 4). In no case did the rise in thoracic duct lymph iron exceed 300 micrograms per cent. These experiments serve to show rather conclusively that resorption of iron from the intestinal tract occurs directly into the blood stream rather than by way of the intermediate route of the intestinal lymph channels.

Rate of disappearance of iron from the blood stream

Before continuing further, it was obviously necessary to know something about the rate at which iron disappears from the blood stream. Starkenstein and Weden (34a) and Barkan (35) have shown that when iron is added to whole blood *in vitro*, all of the added iron can be recovered from the serum portion. We have demonstrated that when iron salts are injected intra-

venously, the only blood iron fraction to show an increase is the serum iron fraction. In order to obtain information as to the rate of disappearance of added iron from the serum therefore, sufficient quantities of various iron salts were injected into dogs to provide one milligram of the metallic metal per kilogram of body weight. The salts were first dissolved in water and then diluted with several parts of normal saline and injected slowly into one of the leg veins. Serum samples were collected immediately before the injection, and 5 minutes, 1 hour, 3 hours, 5 hours, 8 hours and 12 hours after the administration. The animals were kept anesthetized throughout the experimental period.

As is obvious from Figures 5 and 6 one milligram of iron per kilogram of body weight given intravenously in the form of a soluble salt was sufficient to increase the level of serum iron from an initial value of about 0.2 mgm per cent to from 1.2 to 1.5 mgm per cent. The subsequent fall was a gradual one so that about 12 hours were required before the basal level was again approximated. There was apparently no difference in the rapidity with which ferric iron given as a simple (ferric chloride) or combined (ferric ammonium sulphate) salt was removed (Figure 5).

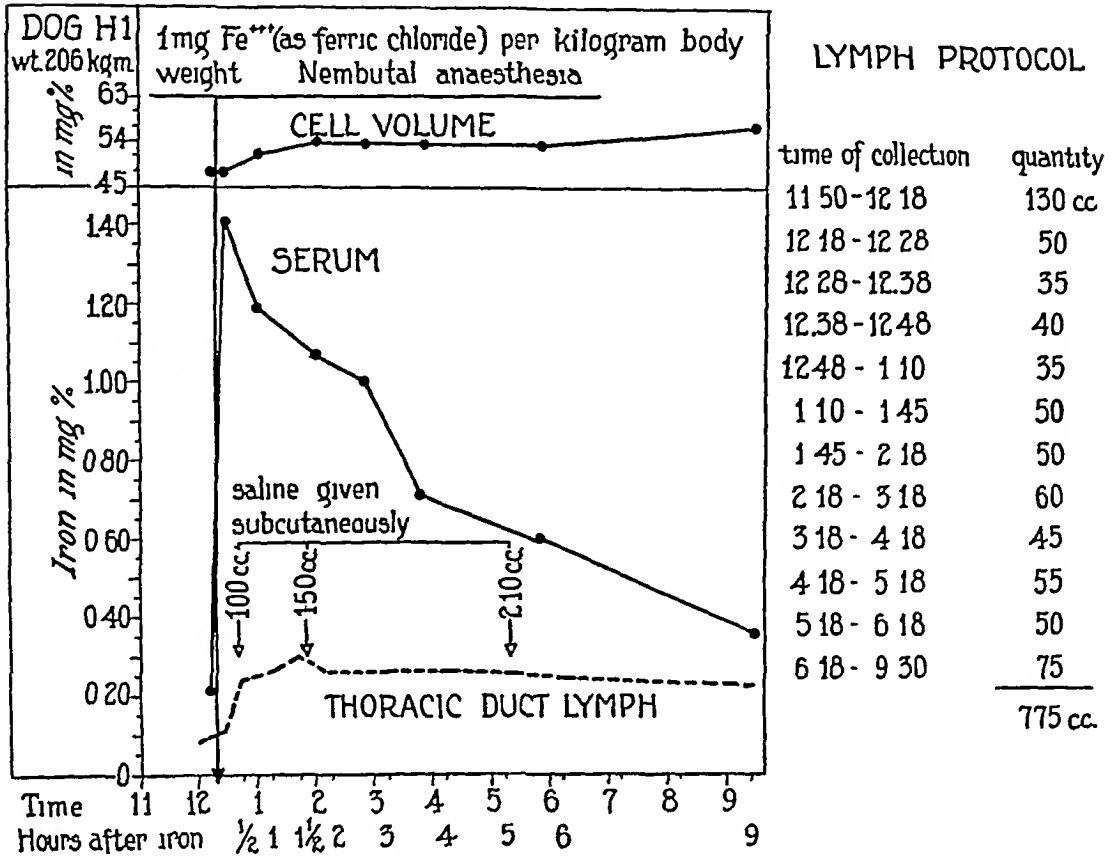


FIG 4 APPEARANCE OF IRON IN THORACIC DUCT LYMPH AFTER INTRAVENOUS ADMINISTRATION OF IRON TO A DOG

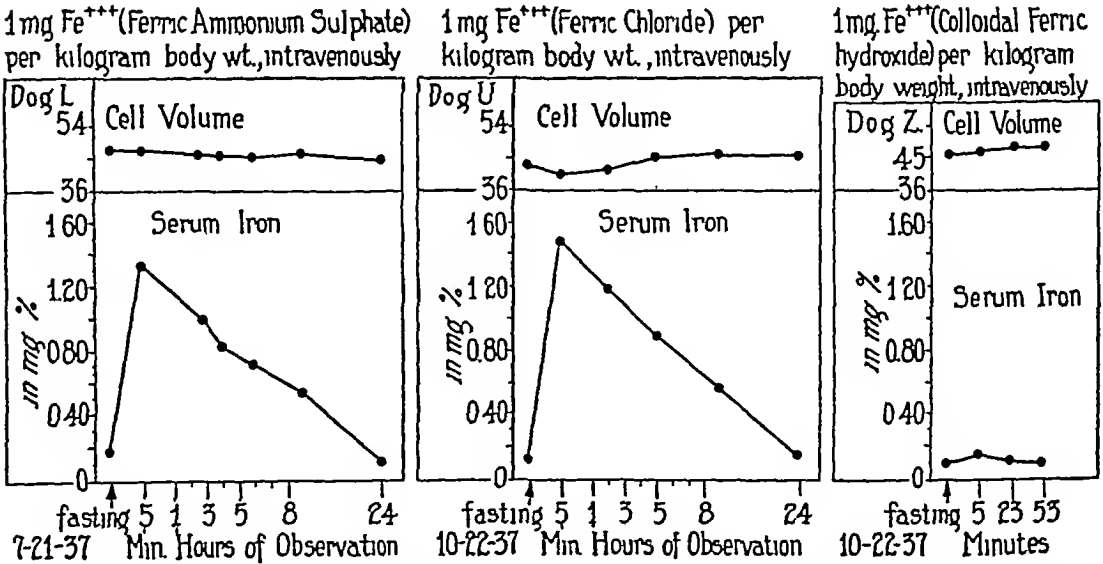


FIG 5 COMPARISON OF RATES OF DISAPPEARANCE OF VARIOUS FORMS OF FERRIC IRON FROM THE BLOOD STREAM AFTER INTRAVENOUS ADMINISTRATION

1mg Fe as ferrous and ferric ammonium sulphate per kilogram body weight. 1mg Fe as ferrous and ferric chloride per kilogram body weight. 1mg Fe as ferrous sulphate and ferric chloride per kilogram body weight

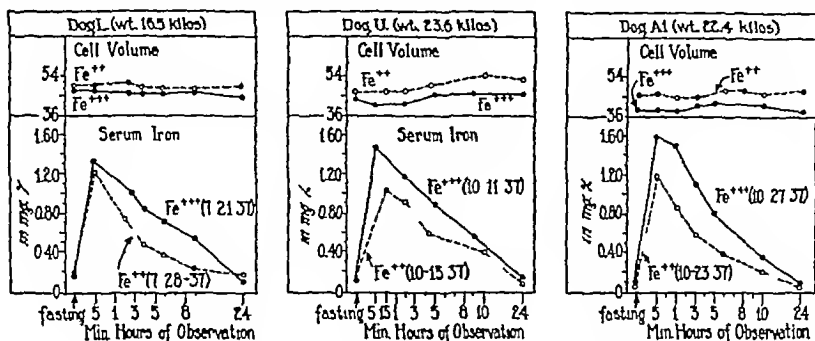


FIG. 6. COMPARISON OF RATES OF DISAPPEARANCE OF FERROUS AND FERRIC IRON FROM THE BLOOD STREAM AFTER INTRAVENOUS ADMINISTRATION

Nembutal anaesthesia used throughout duration of experiment. Iron administered intravenously in each instance immediately after fasting specimen.

When colloidal ferric hydroxide was used, however, the immediate rise was comparatively small and the rate of disappearance rapid enough to be complete within 10 to 15 minutes. Thus result is not surprising since it has been known for a long time that colloidal ferric hydroxide is taken up by the reticulo-endothelial cells (36 37). In a number of animals the fall in serum iron following the administration of comparable amounts of soluble ferrous and ferric iron to the same animal was followed (Figure 6). In each instance, irrespective of whether simple or combined salts were used, the height to which the serum iron values rose was less with the ferrous salts than with the ferric. The explanation for this phenomenon is not clear, since Starkenstein and Harvalik (38) have shown that ferrous iron is almost immediately oxidized to the ferric state on contact with whole blood.

Height of serum iron response compared with amount of iron administered

The most obvious objection to the use of the serum iron increase as an index of intestinal absorption of iron is that iron is a protein precipitant and for that reason may cause enough injury to the intestinal mucosa to permit a portion

of the large amounts administered to pass through into the blood stream. In order to test the validity of this objection and to establish at the same time the size of the dose that could best be used for future comparisons, several observations were performed on the relationship between the height of the serum iron increase and the size of the iron dose. In one such instance the subject being a young male with normal gastric acidity single doses of ferric sodium citrate were given on each of 4 different observation days in quantities that supplied successively 2.7, 5.4, 8.1, and 10.7 mgm. of iron per kilogram of body weight (Figure 7). Serum specimens were collected immediately before the iron was ingested, and at approximately 1 1/2, 5, 8, and 12 hours thereafter. This routine of blood collection was followed for all the studies made on human patients except that the 12 hour specimen was occasionally omitted. The response to 2.7 mgm. of iron per kilo was approximately one half the response to 5.4 mgm. and one-third of that to 8.1 mgm. per kilo. With the largest dose 10.7 mgm. per kilo, considerable abdominal discomfort was experienced and a diarrhea developed within a few hours. It is interesting to note that the maximum increase above the initial level fol-

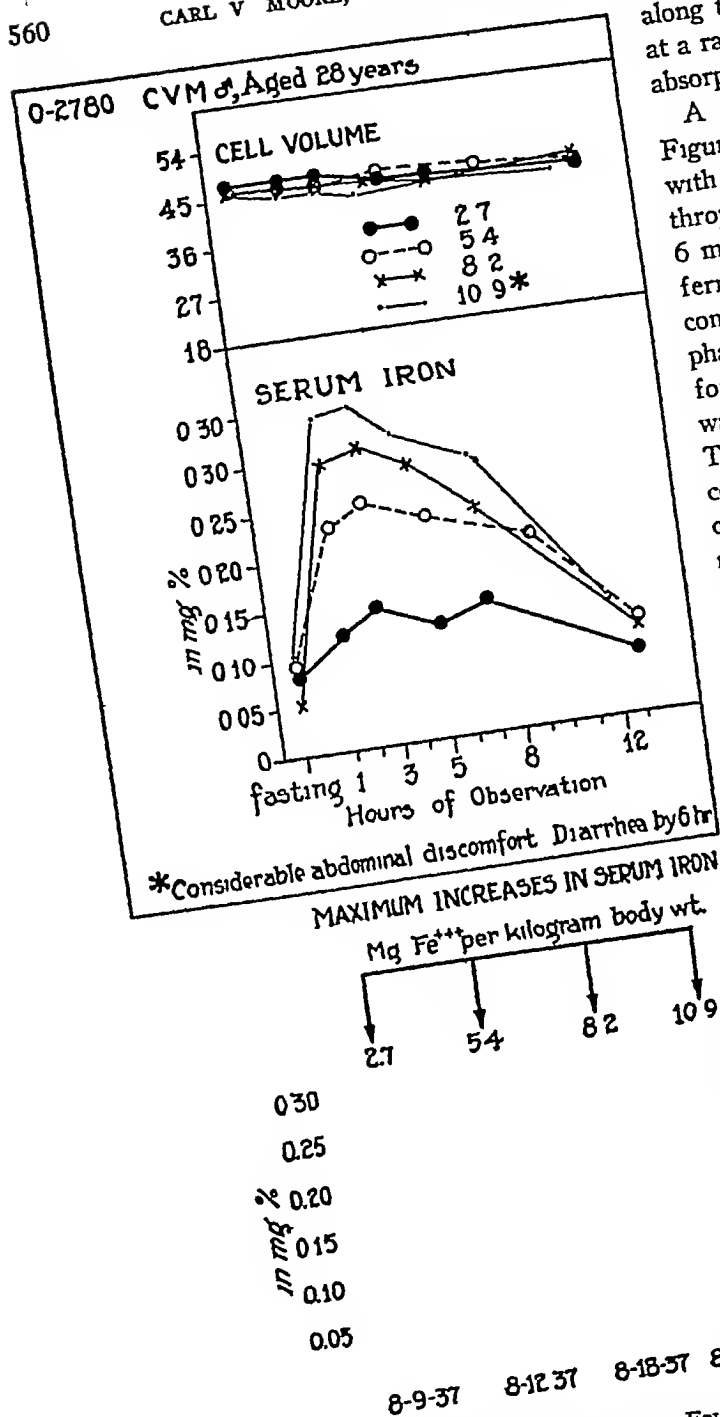


FIG 7 RISE IN SERUM IRON VALUES FOLLOWING SINGLE ORAL DOSES OF FERRIC SODIUM CITRATE IN A "NORMAL" SUBJECT

lowing this amount was no greater than it had been after the 8.1 mgm dosage. It is probable that the increased intestinal irritation was attended by increased motility so that the iron was swept

along the absorbing portion of the small intestine at a rate that was too rapid to permit of optimum absorption

A similar set of observations is tabulated in Figure 8. The subject was again a young male with normal gastric acidity and undisturbed erythropoiesis. He was given successively 2, 4, and 6 mgm of iron per kilogram of body weight as ferric ammonium sulphate on each of 3 days, and comparable dosages of ferrous ammonium sulphate on 3 additional days. With both valence forms, the response to the 4 mgm per kilo dose was approximately twice that to half the amount. The largest dose of iron, however, again caused considerable gastric distress and abdominal cramps, and again the serum iron increase was not directly proportional to the quantity given. The rise in serum iron following any given iron administration, therefore, is apparently roughly proportional to the amount of the metal administered, up to that point at which the intestinal irritation becomes great enough to alter motility and to interfere with the absorptive process.

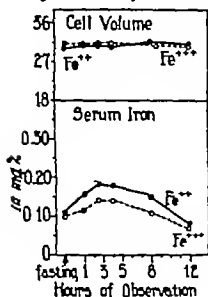
Relative constancy of serum iron rise produced by ingestion of identical amounts of ferrous sulphate in the same subject

As another control of the applicability of this method to the study of iron absorption, it was necessary to determine how constant the serum iron increase produced by a given dose of iron might be in any particular subject. The individuals who volunteered for this study were afebrile and in apparent hematologic equilibrium. Ferrous sulphate was arbitrarily chosen as the salt to be used and was given in identical amounts on each of 3 or 4 different observation days. The absorption curves produced closely paralleled each other in every subject (Figures 9, 10, 12, 13, 19) and were used as a standard of reference for comparing the relative degrees of absorption produced by other iron salts or by ferrous sulphate under the influence of certain specific factors to be described later. In order to make such a comparison easier, the limits of variation in response to these several administrations of ferrous sulphate were drawn as striated areas on the subsequent charts.

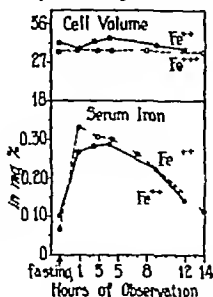
O 3757 CF ♂, Aged 32 years

Normal Gastric Acidity

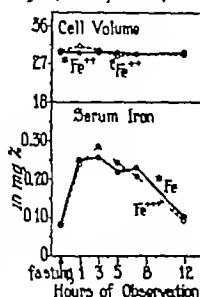
2 mg. Fe per kilogram body wt.



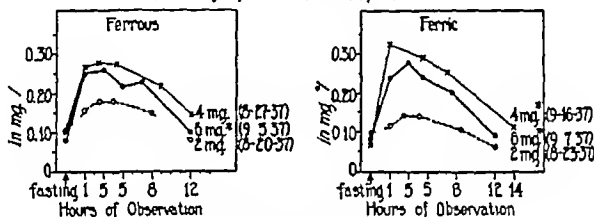
4 mg. Fe per kilogram body wt.



6 mg. Fe per kilogram body wt.



Summary of Serum Iron Responses



Ferrous iron given as ferrous ammonium sulphate

Ferric iron given as ferric ammonium sulphate

*Gastric distress, abdominal cramps and diarrhea following administration.

FIG. 8. INCREASES IN SERUM IRON VALUES FOLLOWING ORAL ADMINISTRATION OF FERROUS AND FERRIC AMMONIUM SULPHATE IN A "NORMAL" SUBJECT

Comparison of the serum iron absorption curves produced by various of the water soluble ferrous salts

Starkenstern in 1928 (39) suggested that both ferrous and ferric iron were absorbed from the intestinal tract and that ferrous sulphate was re-sorbed less readily than was ferrous chloride. The anion was thought to be the determining factor in causing the differences in rate of absorption. Wallbach (40) using a histological technique, came to essentially the same conclusion that the anion to which iron was bound was of greater importance than solubility of the salt or chemical properties of the metallic portion of the molecule.

This question seemed an ideal one for study

by means of serum iron absorption curves. Comparable amounts of various ferrous salts were given to a number of subjects both with and without normal gastric acidity. Ferrous chloride, ferrous citramate, ferrous gluconate, ferrous carbonate and ferrous ammonium sulphate were found to give serum iron rises comparable to those produced by ferrous sulphate both in individuals with a normal amount of free HCl in their gastric contents and in those with a histamine refractory achlorhydria (Figures 9, 10, 12, 13). The relatively insoluble ferrous phosphate, on the other hand, caused no appreciable change in serum iron values (Figure 14). Thoenes and Aschaffenburg found that ferrous chloride and a ferrous preparation known as

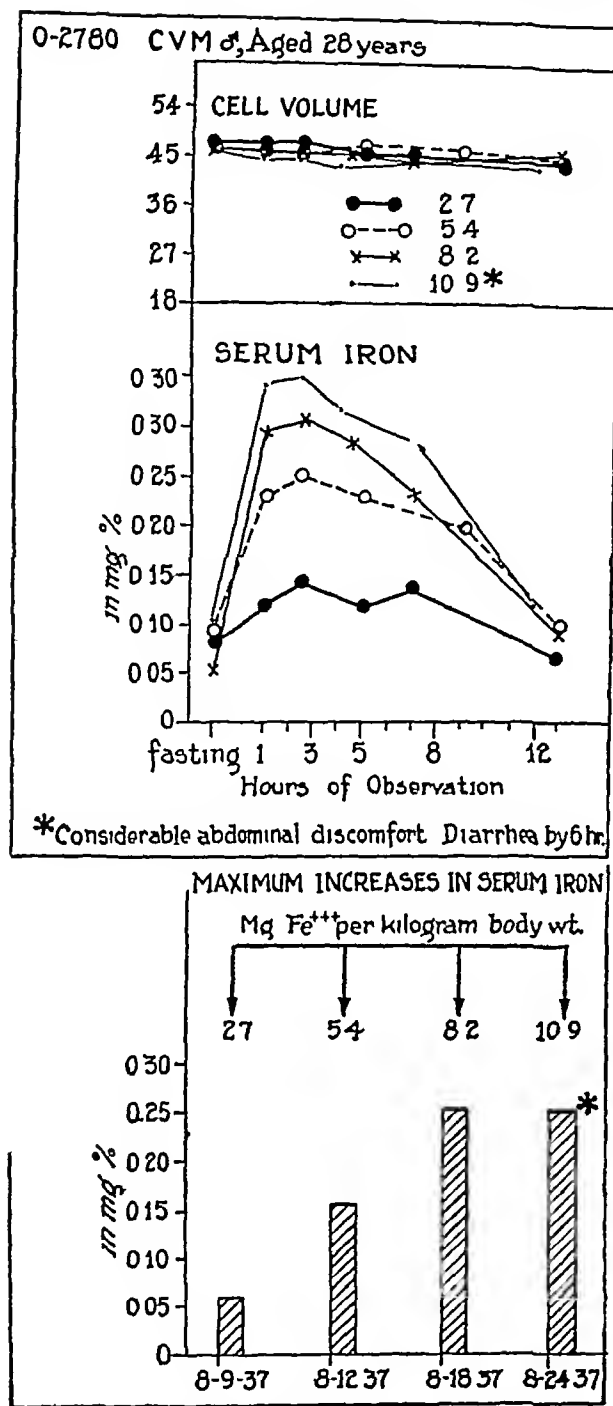


FIG 7 RISE IN SERUM IRON VALUES FOLLOWING SINGLE ORAL DOSES OF FERRIC SODIUM CITRATE IN A "NORMAL" SUBJECT

lowing this amount was no greater than it had been after the 81 mgm dosage. It is probable that the increased intestinal irritation was attended by increased motility so that the iron was swept

along the absorbing portion of the small intestine at a rate that was too rapid to permit of optimum absorption.

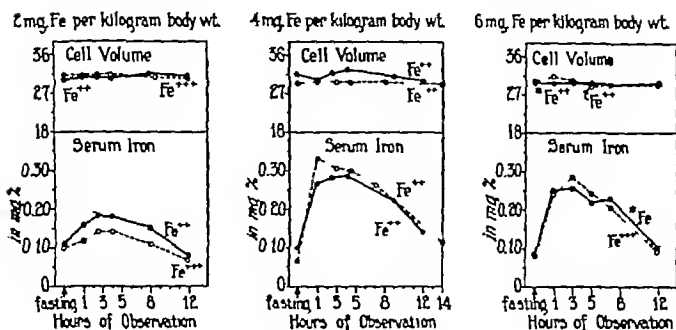
A similar set of observations is tabulated in Figure 8. The subject was again a young male with normal gastric acidity and undisturbed erythropoiesis. He was given successively 2, 4, and 6 mgm of iron per kilogram of body weight as ferric ammonium sulphate on each of 3 days, and comparable dosages of ferrous ammonium sulphate on 3 additional days. With both valence forms, the response to the 4 mgm per kilo dose was approximately twice that to half the amount. The largest dose of iron, however, again caused considerable gastric distress and abdominal cramps, and again the serum iron increase was not directly proportional to the quantity given. The rise in serum iron following any given iron administration, therefore, is apparently roughly proportional to the amount of the metal administered, up to that point at which the intestinal irritation becomes great enough to alter motility and to interfere with the absorptive process.

Relative constancy of serum iron rise produced by ingestion of identical amounts of ferrous sulphate in the same subject

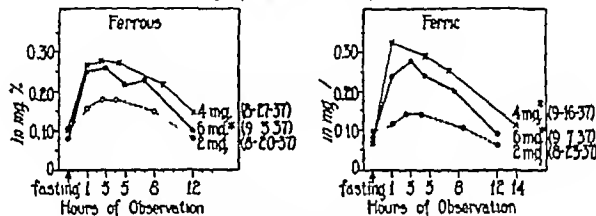
As another control of the applicability of this method to the study of iron absorption, it was necessary to determine how constant the serum iron increase produced by a given dose of iron might be in any particular subject. The individuals who volunteered for this study were afebrile and in apparent hematologic equilibrium. Ferrous sulphate was arbitrarily chosen as the salt to be used and was given in identical amounts on each of 3 or 4 different observation days. The absorption curves produced closely paralleled each other in every subject (Figures 9, 10, 12, 13, 19) and were used as a standard of reference for comparing the relative degrees of absorption produced by other iron salts or by ferrous sulphate under the influence of certain specific factors to be described later. In order to make such a comparison easier, the limits of variation in response to these several administrations of ferrous sulphate were drawn as striated areas on the subsequent charts.

O 3757 C.F. of, Aged 32 years.

Normal Gastric Acidity



Summary of Serum Iron Responses



Ferrous iron given as ferrous ammonium sulphate

Ferric iron given as ferric ammonium sulphate

*Gastric distress abdominal cramps and diarrhea following administration.

FIG. 8. INCREASES IN SERUM IRON VALUES FOLLOWING ORAL ADMINISTRATION OF FERROUS AND FERRIC AMMONIUM SULPHATE IN A "NORMAL" SUBJECT

Comparison of the serum iron absorption curves produced by various of the water soluble ferrous salts

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This question seemed an ideal one for study

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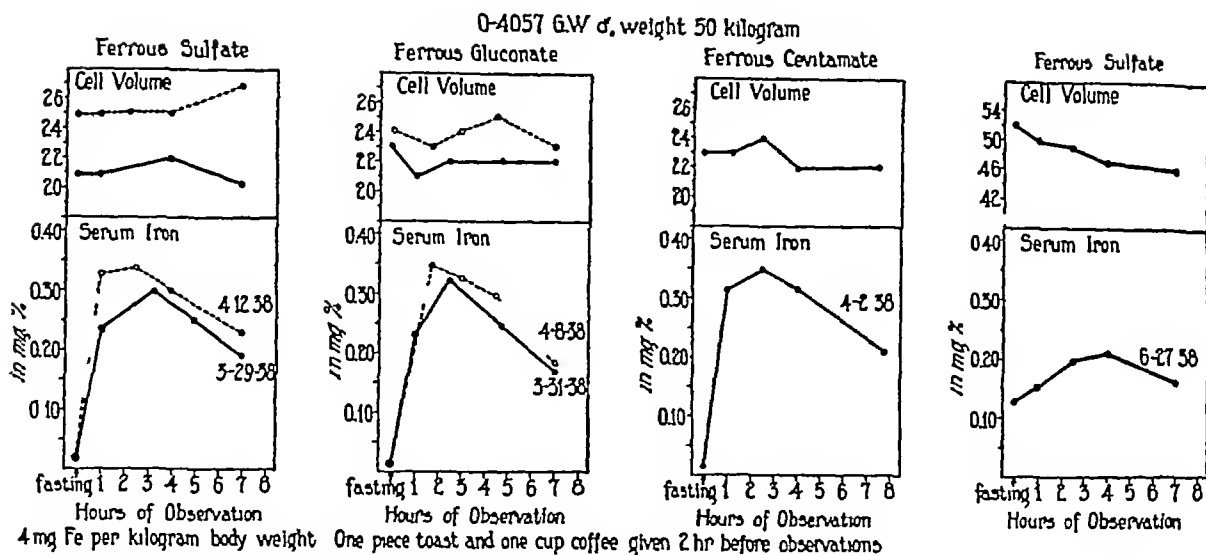


FIG 9 SERUM IRON INCREASES IN AN ADULT MALE WITH A HYPOCHROMIC, MICROCYTIC ANEMIA AND NORMAL GASTRIC ACIDITY

O-4080 H M C D ♂, Aged 30 years, weight 67.7 kilogram Normal Gastric Acidity

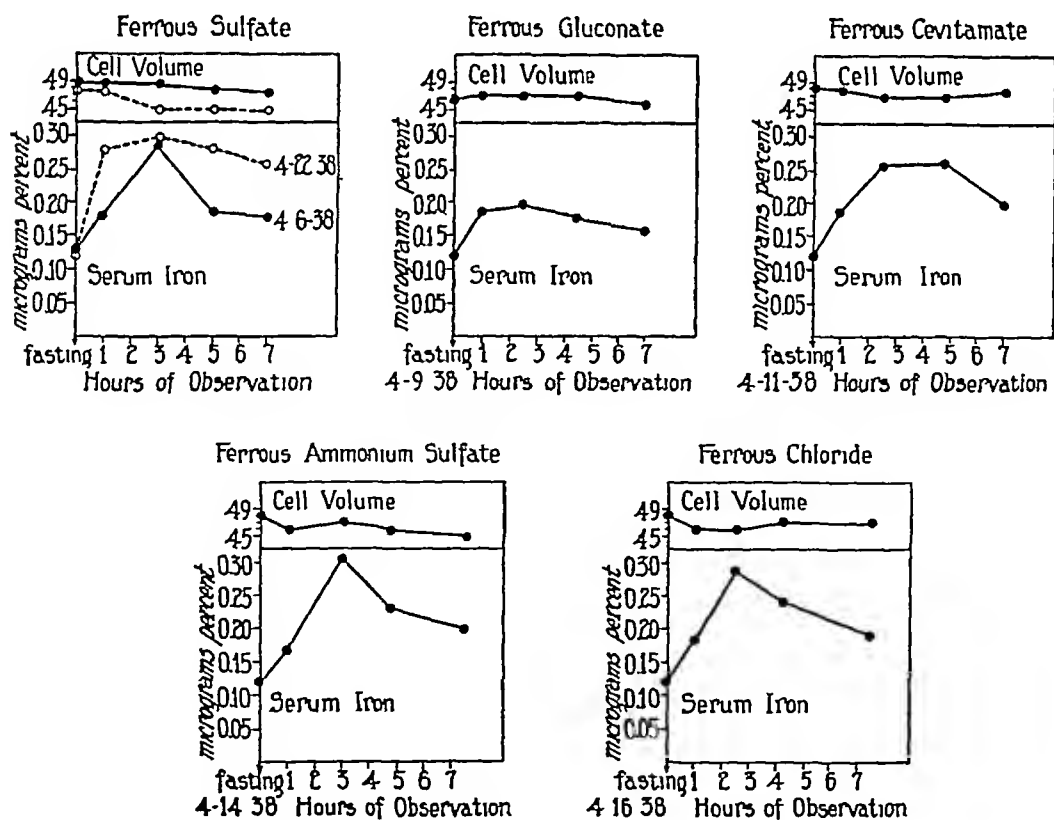


FIG 10 COMPARISON OF SERUM IRON CURVES PRODUCED BY COMPARABLE AMOUNTS OF VARIOUS FERROUS SALTS IN A SUBJECT WITH NORMAL GASTRIC ACIDITY AND NORMAL HEMATOLOGICAL FINDINGS

"ferrostabil" (stabilized ferrous chloride) were capable of producing greater serum iron increases than was ferrum reductum in comparable amounts (27). These results suggest that ease of ionization of the ferrous salts, under those conditions of acidity or alkalinity which prevail in the gastro-intestinal tract is the important factor in determining their relative rates of absorption. The nature of the anion, except as it influences this factor, would seem to be relatively unimportant.

Comparison of serum iron absorption curves produced by comparable amounts of ferrous and ferric salts of the same anion

There is an increasing volume of experimental evidence which tends to indicate that iron is absorbed from the upper portion of the small intestine as ferrous iron (32, 34b, 41, 42). That the evidence is not entirely convincing is indicated by recently expressed dissenting views (15, 18, 22). However, the concept is in accord with the repeatedly demonstrated clinical fact that remissions from iron deficiency anemias, in the human at least can be produced by smaller amounts of soluble ferrous salts than of soluble

ferric preparations (for bibliography, consult review by Heath and Patek—reference 11).

In order to study the question further, serum iron increases produced by the oral administration of comparable amounts of ferrous and ferric salts of the same anions were determined. Heilmeyer and Plötner in 1936 (28a) reported that much greater rises in serum iron were observed following the ingestion of ferrous iron than of similar quantities of ferric. In the great majority of instances, we obtained the same result. Rises in serum iron effected by ferric salts were either negligible or definitely lower in the quantities used than those obtained following ferrous preparations (Figures 11, 15, 16, 21). The data charted in Figure 12 serve as an excellent example of these findings and permit comparison of the curves for both simple (ferrous and ferric sulphate) and combined (ferrous and ferric ammonium sulphate) salts. It is interesting to note that 12 mgm of iron per kilogram of body weight as iron and ammonium citrate were necessary to produce an increase in the serum iron curve comparable to that produced by one third the quantity of iron as ferrous sulphate.

Occasionally, however, responses following

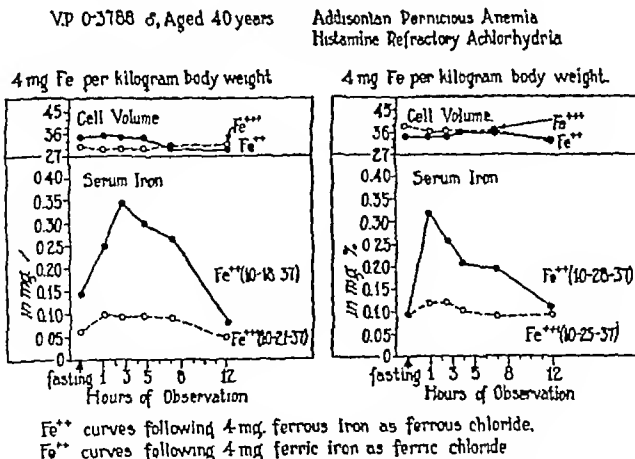


FIG. 11. SERUM IRON INCREASES FOLLOWING SINGLE ORAL DOSES OF FERROUS AND FERRIC CHLORIDE IN A SUBJECT WITH HISTAMINE REFRACTORY ACHILYDRIA

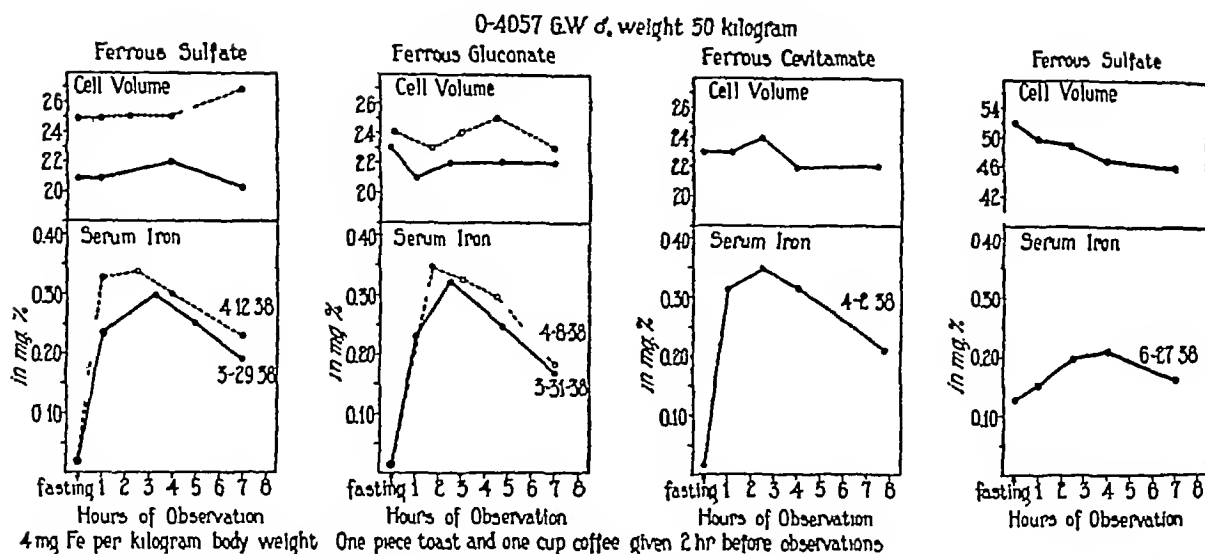


FIG 9 SERUM IRON INCREASES IN AN ADULT MALE WITH A HYPOCHROMIC, MICROCYTIC ANEMIA AND NORMAL GASTRIC ACIDITY

O 4080 H M^cD ♂, Aged 30 years, weight 677 kilogram Normal Gastric Acidity

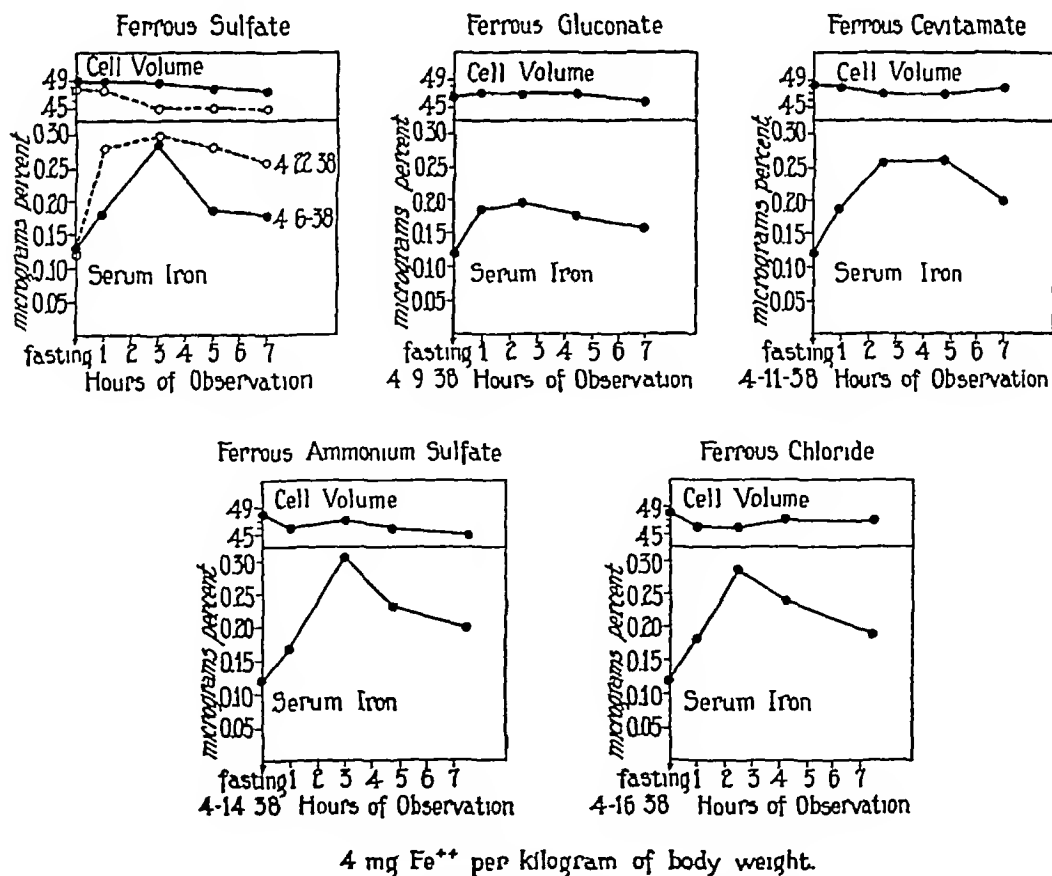


FIG 10 COMPARISON OF SERUM IRON CURVES PRODUCED BY COMPARABLE AMOUNTS OF VARIOUS FERROUS SALTS IN A SUBJECT WITH NORMAL GASTRIC ACIDITY AND NORMAL HEMATOLOGICAL FINDINGS

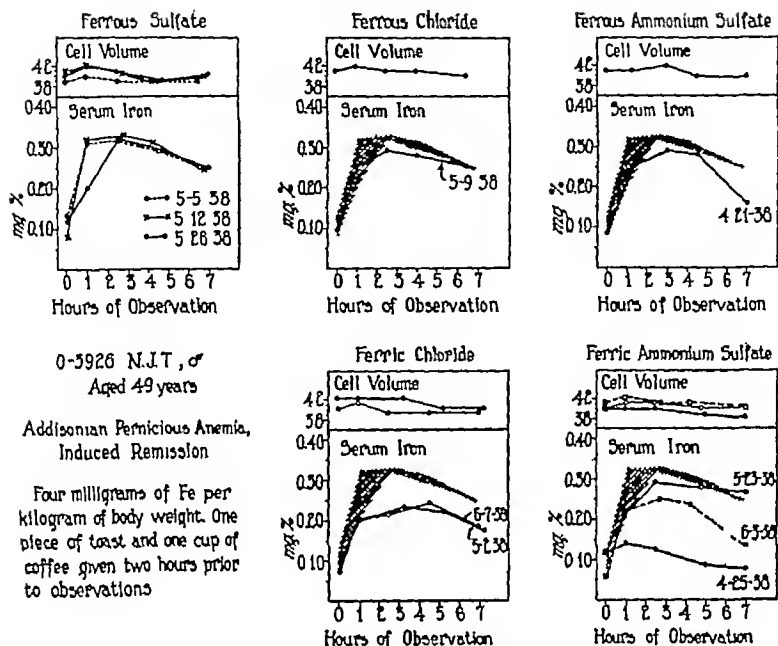


FIG 13 INCREASES IN SERUM IRON FOLLOWING THE ORAL ADMINISTRATION OF INORGANIC SOLUBLE FERROUS AND FERRIC SALTS TO A PATIENT WITH HISTAMINE REFRACTORY ACHLOHYDRIA

ference in the degree of absorption produced at different times by identical amounts of the same ferric salt is not immediately obvious unless it is assumed (1) that it is necessary for ferric iron to be reduced to the bivalent state before it can be absorbed and (2) that there was a corresponding variation in the reduction potentialities of the small intestine at the time of these 3 trials

The fact that ferrous salts produced higher rises in serum iron than were usually observed following the oral administration of comparable amounts of corresponding ferric salts adds further weight to the conviction that iron is absorbed from the intestinal tract in a bivalent form. The following observations were even more convincing. To a number of subjects who had failed to show an increase in serum iron following ferric salts, the iron administration was

supplemented with either 10 gram of cevitamic acid or 15 grams of sodium formaldehyde sulfoxylate as reducing substances. Invariably a good response was obtained the height of the curves occasionally being higher than those produced by the ferrous salt of the same anion (Figures 15 16). This latter observation led us to compare the degree of absorption of ferrous salts when given alone with that which occurred when they, too, were supplemented with one of the reducing substances just mentioned. Usually, the serum iron increase was greater with cevitamic acid or sodium formaldehyde sulfoxylate than without it. Negative results were obtained on several patients with two other reducing substances glutathione in half gram doses (Figures 18, 19) and methylene blue in gram amounts. Cysteine was not tried. The effectiveness of cevitamic acid in increasing absorption of ferric

0-3987 C B ♂, Aged 28 years, weight 73 kilo

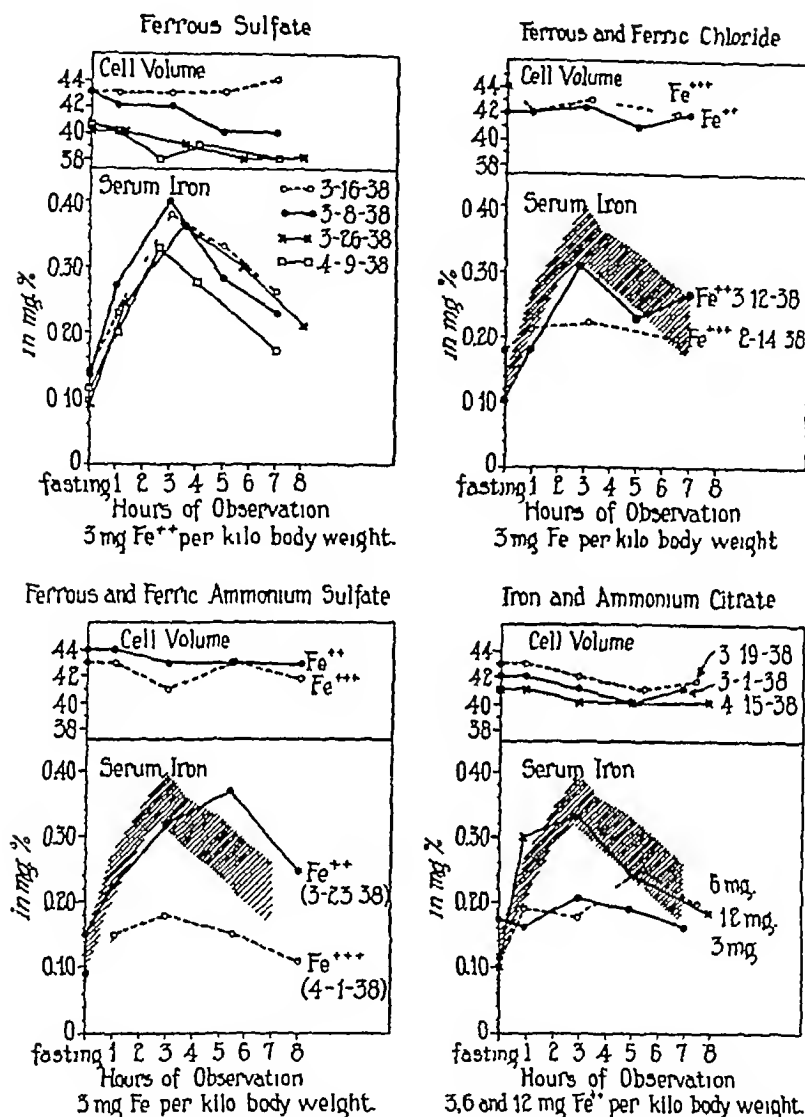


FIG 12. COMPARISON OF SERUM IRON CURVES PRODUCED BY COMPARABLE AMOUNTS OF VARIOUS IRON SALTS IN A SUBJECT WITH NORMAL GASTRIC ACIDITY AND NORMAL HEMATOLOGICAL FINDINGS

ferric iron administration were equally as good as those which followed ferrous iron. One example of that finding has already been presented in Figure 8, another is recorded in Figure 17. Of particular interest in this respect are the observations on N J T, a white male, 49 years of age, who had a histamine refractory achlorhydria (Figure 13). He was given ferric chloride on each of 2 days. The absorption curves produced agreed well with each other and were definitely lower than those produced by compar-

able dosages of ferrous sulphate, ferrous chloride, or ferrous ammonium sulphate. When ferric ammonium sulphate was used, however, there was practically no rise in the serum iron level during the first day, as great a response as was produced by the ferrous salts during the second, and a moderate increase during the third day of observation. The patient had been permitted to take a breakfast of one piece of toast and one cup of black unsweetened coffee two hours before each of the tests was begun. The reason for the dif-

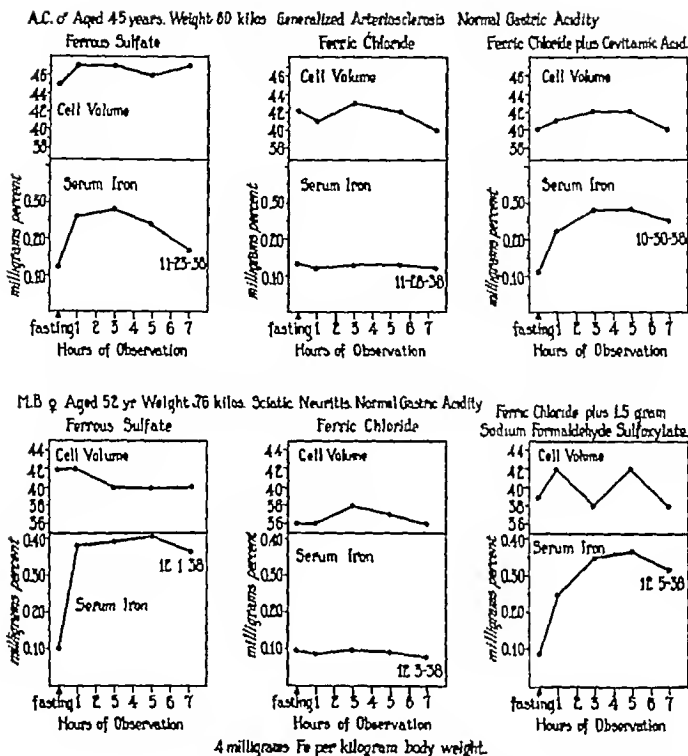


FIG. 15 EFFECT OF CEVITAMIC ACID AND SODIUM FORMALDEHYDE SULFOXYLATE ON SERUM IRON ABSORPTION CURVES

the organism. It is to be emphasized again that the failure of a particular iron preparation to cause an increase in the serum iron level does not necessarily mean that none of the metal was absorbed. Such a result indicates only that the rate and degree of absorption were not great enough to add iron to the blood stream more rapidly than the combined organs of storage, utilization and excretion could remove it.

Effect of gastric acidity on the serum iron responses to orally administered ferrous and ferric salts

While it is generally admitted that normal gastric acidity is necessary for optimal assimilation

of food iron (32, 41, 42, 48, 49, 50), there is some dispute as to its effect upon the absorption of metallic iron salts in therapeutic doses. Mettler and Minot (51) obtained a greater reticulocyte peak when ferric ammonium citrate was given to patients with a hypochromic anemia in an acid buffered solution than when given in an alkali buffered medium. The observation was confirmed by Heath (17) and by Minot and Heath (52). Brock and Taylor (53) and Lintzel (32) have shown that the dialysis of soluble inorganic iron salts is hastened by the addition of small amounts of dilute mineral acids and hindered when the solutions are made weakly alkaline. These findings are in accord with those

salts in patients with achlorhydria had previously been noted by Heilmeyer (43), who thereupon introduced ferrous cevitamate as a useful form of bivalent iron for therapeutic use. It is reasonable to postulate that, in the case of ferrous salts, cevamic acid and sodium formaldehyde sulfoxylate aid in preventing partial conversion to the ferric state, and, in the case of ferric salts, aid the reducing mechanisms of the small intestine to make reduction more complete.

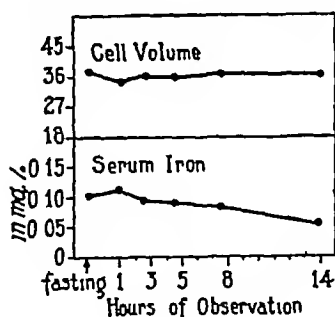
When the relatively insoluble ferric phosphate was given by mouth, no increase in serum iron occurred (Figure 14). It has already been indicated that the same was true for ferrous phosphate.

These results add confirmatory data to the mass of experimental and clinical evidence already accumulated which indicates that iron is absorbed from the intestinal tract more readily, if not entirely, in the bivalent state. Serum iron absorption curves were higher as a rule following the

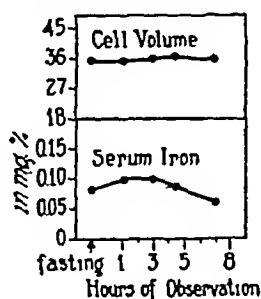
ingestion of ferrous salts than of ferric. Frequently, the latter failed to cause any detectable rise in the serum iron fraction whatever. This decreased effectiveness of the ferric salts is in accord with the idea that highly ionized ferric compounds are changed rapidly in the neutral or alkaline medium of the upper small intestines (44) into insoluble ferric hydroxide and other insoluble or non-diffusible ferric compounds (45, 46, 47). Certain reducing substances, notably cevamic acid and sodium formaldehyde sulfoxylate, when given orally along with iron, increased the height of the serum iron curves as produced either by ferrous or ferric iron. This suggests that in those instances in which ferric salts caused an appreciable increase in serum iron, the reducing mechanisms in the small intestine were efficient enough to effect a reduction to the ferrous state before great quantities of the ingested compound had been converted to non-ionizable forms, and hence removed from availability to

0-3313 SB ♂, Aged 77 years. Addisonian Pernicious Anemia
Histamine Refractory Achlorhydria

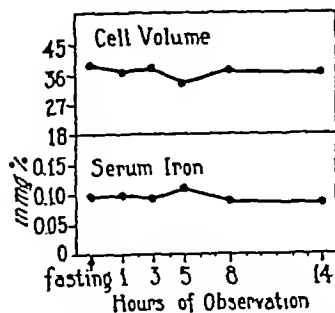
0.75 gm. Fe^{++} as Ferrous Phosphate (6.76 gm)



0.75 gm Fe^{++} as Ferrous Phosphate (6.76 gm)
plus 100 cc. 0.1N. HCl



0.75 gm. Fe^{+++} as Ferric Phosphate (3 gm)



0.75 gm Fe^{+++} as Ferric Phosphate (3 gm)
plus 100 cc. 0.1N. HCl

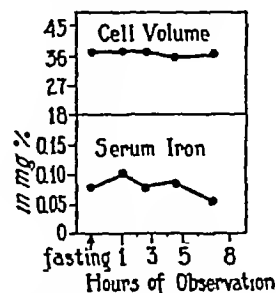
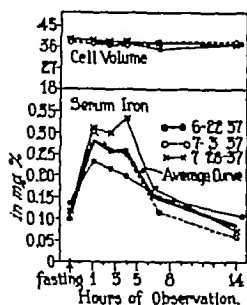


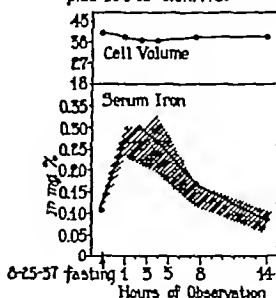
FIG 14 SERUM IRON VALUES FOLLOWING ORAL INGESTION OF SINGLE DOSES OF FERROUS AND FERRIC PHOSPHATE

O-5515 S.B. ♂, Aged 77 years. Addisonian Pernicious Anemia.
Histamine Refractory Achlorhydria.

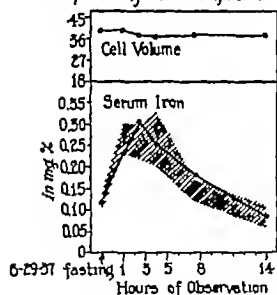
0.75 gm. Fe^{++} as Ferric Sodium Citrate



0.75 gm. Fe^{++} as Ferric Sodium Citrate.
plus 100 cc. 0.1N. HCl



0.75 gm. Fe^{++} as Ferric Sodium Citrate
plus 3 gm. Bile Pigment.



0.75 gm. Fe^{++} as Ferrous Ammonium Sulphate.

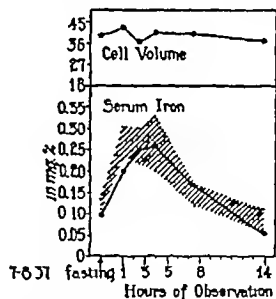


FIG. 17 STUDY OF SERUM IRON INCREASES FOLLOWING SINGLE ORAL DOSES OF IRON SALTS IN A PATIENT WITH ACHLORHYDRIA

in subjects with achlorhydria (Figure 16) just as in patients with normal acidity. The effects with ferric salts were occasionally less dramatic (Figure 16). Attention is called to the fact that the amounts of iron used in these tests were relatively large.

The apparent discrepancy between our results and those of Heilmeyer and Plötnner can readily be explained. These latter workers used reduced iron, a form which must be dissolved and ionized in the gastro-intestinal tract before it can be absorbed. Free hydrochloric acid in the gastric contents undoubtedly aids that process. In this respect, the role which gastric acidity plays is

similar to that for food iron. We, on the other hand, used highly ionized water soluble salts. The effect of gastric acidity on these preparations is apparently to delay the formation of insoluble compounds (at a pH above 5) until they are brought into contact with the reducing forces of the small intestine. The salts we used however were not only acid in reaction but were used in such comparatively large amounts that they almost certainly reached the small intestine before neutralization had taken place. For this reason this method of studying iron absorption by the serum iron curve technic is probably not particularly applicable to the analysis of the role which

of Halvorsen and Starkey who, working with pure solutions of iron, noted that at a pH above 5.0 only small concentrations of ferrous, and still smaller concentrations of ferric, iron are present in solution (45). It has been emphasized that the normal acidity of the stomach and duodenal contents is favorable to the formation and preservation of ferrous ions in solution and delays the change into insoluble or undissociated, particularly ferric, compounds (32, 41, 42, 46, 47). Nevertheless, good hematologic responses are constantly observed following iron therapy for hypochromic anemia in individuals with complete anacidity, and Barer and Fowler (50) obtained as much retention of iron, when the intake was high, in patients with an achlorhydria as in those with normal gastric acidity.

The problem has already been studied in part with the serum iron absorption curve technic by Heilmeyer and Plotner (28a). These workers

noted that when 1 gram of reduced iron was given orally, practically no increase in serum iron occurred in patients with achylia gastrica while an appreciable rise was observed in normal subjects. Our investigations have, for the most part, yielded negative findings. The height of the absorption curves produced by the ingestion of ferrous salts was usually as great in individuals with achlorhydria as in those with normal gastric acidity (Figures 11, 13), occasionally, a smaller rise was obtained (Figure 16). As with normal subjects, ferric salts produced significant serum iron increases in rare instances when achlorhydria was present (Figures 13, 17). Addition of 100 cc of 0.1 N HCl to the iron just before its administration failed to increase the height of the absorption curve (Figure 17). If either sodium formaldehyde sulfoxylate or cevitic acid was given just prior to the iron, increases in the serum iron curves were obtained

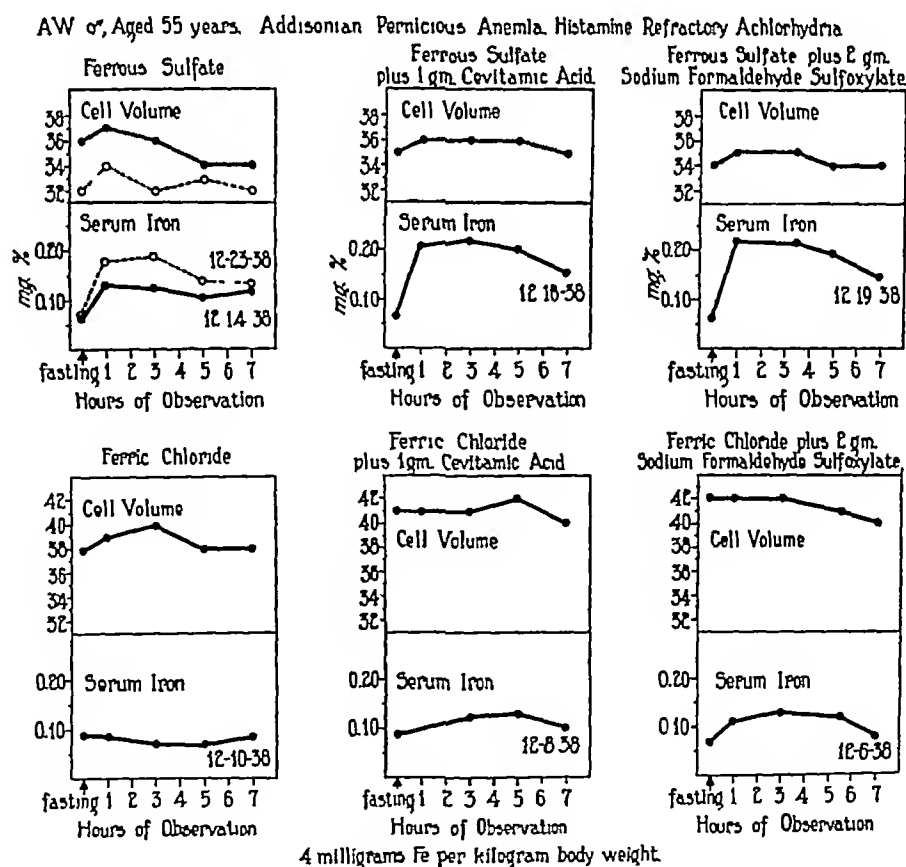
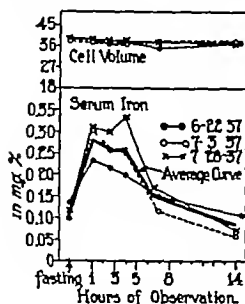


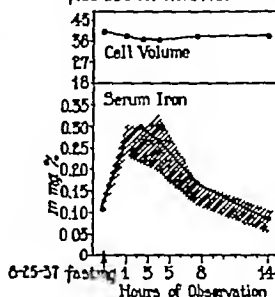
FIG 16 EFFECT OF CEVITAMIC ACID AND SODIUM FORMALDEHYDE SULPHOXYLATE ON SERUM IRON ABSORPTION CURVES

0-3315 S.B. ♂, Aged 77 years. Addisonian Pernicious Anemia.
Histamine Refractory Achlorhydria.

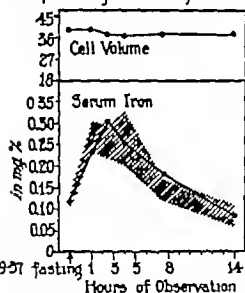
0.75 gm. Fe^{++} as Ferric Sodium Citrate



0.75 gm. Fe^{++} as Ferric Sodium Citrate
plus 100 cc. 0.1N. HCl



0.75 gm. Fe^{++} as Ferric Sodium Citrate
plus 3 gm. Bile Pigment.



0.75 gm. Fe^{++} as Ferrous Ammonium Sulphate

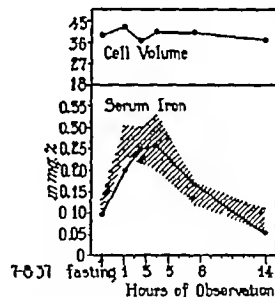


FIG. 17 STUDY OF SERUM IRON INCREASES FOLLOWING SINGLE ORAL DOSES OF IRON SALTS IN A PATIENT WITH ACHLORHYDRIA

in subjects with achlorhydria (Figure 16) just as in patients with normal acidity. The effects with ferric salts were occasionally less dramatic (Figure 16). Attention is called to the fact that the amounts of iron used in these tests were relatively large.

The apparent discrepancy between our results and those of Helmeyer and Plötnner can readily be explained. These latter workers used reduced iron, a form which must be dissolved and ionized in the gastro-intestinal tract before it can be absorbed. Free hydrochloric acid in the gastric contents undoubtedly aids that process. In this respect, the role which gastric acidity plays is

similar to that for food iron. We, on the other hand, used highly ionized water soluble salts. The effect of gastric acidity on these preparations is apparently to delay the formation of insoluble compounds (at a pH above 5) until they are brought into contact with the reducing forces of the small intestine. The salts we used however, were not only acid in reaction but were used in such comparatively large amounts that they almost certainly reached the small intestine before neutralization had taken place. For this reason this method of studying iron absorption by the serum iron curve technic is probably not particularly applicable to the analysis of the role which

gastric acidity plays under conditions of more moderate intake

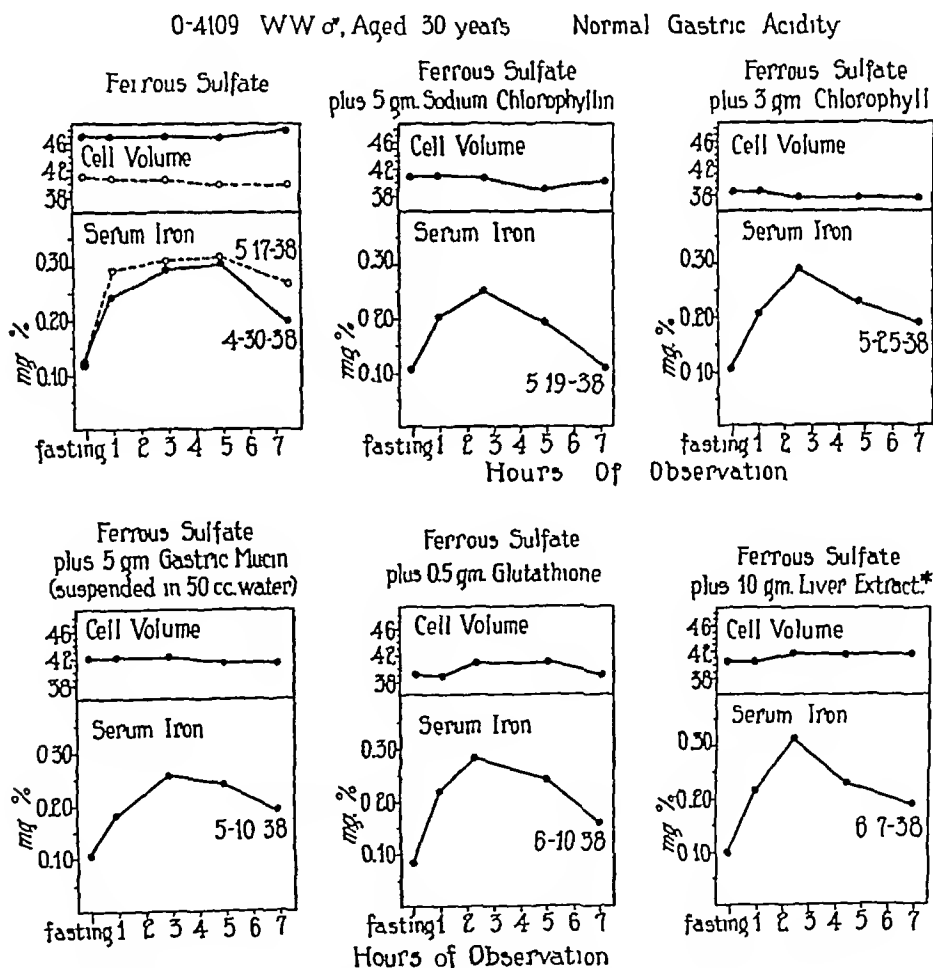
Effect of various miscellaneous substances on the height of serum iron absorption curves

It has been suggested in the recent literature that a number of substances have, in the presence of iron, an additive effect upon hemoglobin production. Patek and Minot (54) described reticulocyte responses to the oral administration of bile pigment following a period of iron medication in patients with hypochromic microcytic anemia. A similar effectiveness for chlorophyll and some of its derivatives was demonstrated by Patek (55). These observations were inter-

preted as suggesting that the body can use pre-formed pyrrol compounds for the building of hemoglobin, no evidence was obtained to indicate that these substances augmented iron absorption. To test that possibility bile pigment,⁵ chlorophyll, and sodium chlorophyllin were given orally along with ferrous sulphate or ferric sodium citrate to several individuals, and the serum iron curves followed. No increase was noted in the height of the curves over that produced by the same amount of the iron alone (Figures 17, 18, 19).

Barker and D. K. Miller administered orally

⁵ The bile pigment used was supplied through the courtesy of Dr. George Minot, Boston.



4 milligrams iron per kilogram body weight *Lilly's Liver Extract #55 without added iron

FIG 18 EFFECT OF VARIOUS SUPPLEMENTARY SUBSTANCES ON THE SERUM IRON INCREASES FOLLOWING THE ORAL ADMINISTRATION OF FERROUS SULPHATE

O 5926 N.J.T. of Aged 49 years. Addisonian Pernicious Anemia, Induced Remission

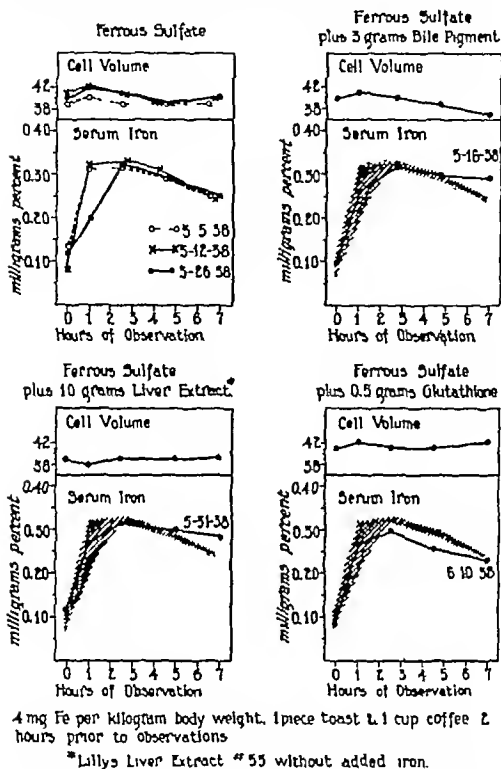


FIG. 19 EFFECT OF BILE PIGMENT LIVER EXTRACT AND GLUTATHIONE ON INCREASES IN SERUM IRON FOLLOWING ORAL ADMINISTRATION OF FERROUS SULPHATE TO A PATIENT WITH HISTAMINE REFRACTORY ACHILORHYDRIA

the secondary anemia liver fraction of Whipple and his coworkers to 11 patients with chronic hypochromic microcytic anemia (56). They concluded that "it appears likely that the liver fraction contains reticulocytogenic material apart from its iron content, and considered it "conceivable that this liver fraction may improve the absorption or utilization of iron. We obtained as had Barker and Miller, the liver fraction Number 55 without added iron from Eli Lilly and Company and gave 10 grams of it by mouth

with ferrous sulphate to several patients. The rise in serum iron produced by this joint administration was no greater than that which occurred following the ingestion of the iron salt alone (Figures 18-19).

Heath Minot, Pohle, and Alstead found that when iron in small doses was administered with relatively large amounts of mucin to cases of chronic hypochromic anemia, its absorption from the intestine was inhibited (57). These workers intentionally used small doses of iron because it

was felt that if large doses had been given, no inhibiting effect of mucin would have been demonstrable. As has already been emphasized many times, with the serum iron absorption curve technique it is necessary to use relatively large amounts of iron. Accordingly, when the effect of 5 grams of "gastric mucin"^a on the absorption curve produced by ferrous sulphate was determined, the amount of the salt used was equivalent to 4 mgm of iron per kilogram of body weight. No alteration in the shape or height of the curve was noted (Figure 18), a fact which confirms the prophesy made by Heath and his coworkers.

The effect of food on the absorption curve of ferrous sulphate was studied in 5 subjects, all of whom had satisfactory serum iron increases when the salt was given after an 18-hour fast. A breakfast of cereal and cream, 2 eggs, 2 strips of bacon, 2 pieces of toast, 1 glass of milk, and 1 cup of coffee was given either immediately before, or immediately after, the ingestion of iron. Two patients, one with normal gastric acidity and the other with a histamine refractory achlorhydria, developed no significant increase in serum iron over the basal level. Two individuals, both with achlorhydria, had a distinct rise in serum iron—but not to the same degree as had occurred following ferrous sulphate alone. The fifth subject, a male with normal gastric acidity, had as great an increase with the food as without it. The reason for this variation in results is not clear. It made no difference whether the breakfast was eaten immediately before, or after, the iron was taken. It may be that in the presence of food, conditions are ripe in the gastro-intestinal tract for the formation of insoluble and undissociable iron compounds. The conclusion is justified that in some individuals, at least, food interferes with the optimal absorption of iron.

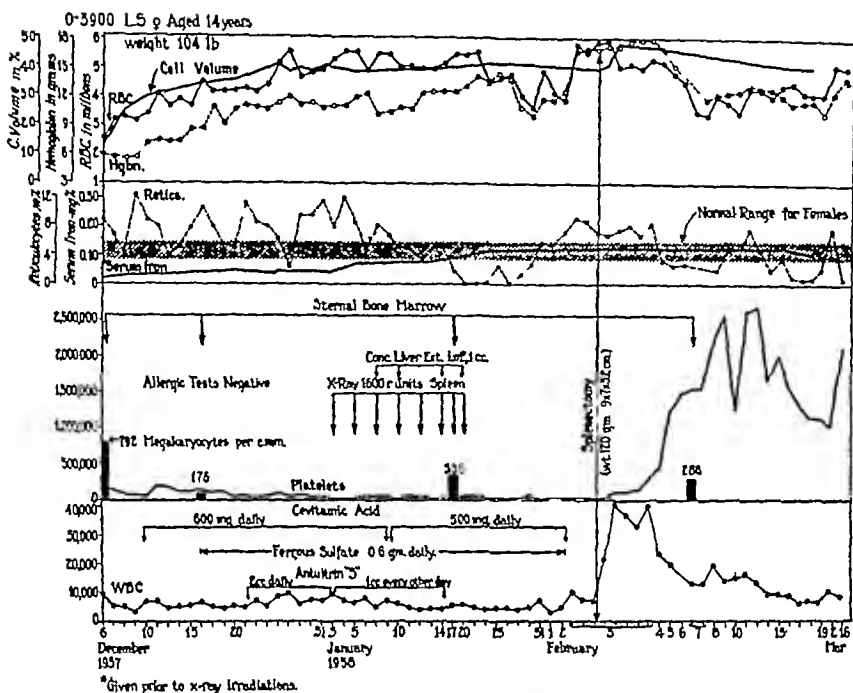
Analysis of the question of selective intestinal absorption of iron

Statement has already been made of the fact that Höber (31), Lintzel (32), and Fontes and Thivolle (33) consider the body capable of assimilating or rejecting iron by a process of se-

lective intestinal absorption. In the presence of an iron deficiency, therefore, more iron should be absorbed than under normal circumstances. Heilmeyer recently studied the serum iron absorption curves produced following the ingestion of 1 gram of ferrum reductum in 2 cases of chronic hypochromic anemia before and after therapy (58). Both of his patients secreted adequate amounts of free hydrochloric acid after an alcohol test meal. In each instance, there was very little evidence of absorption before therapy was begun, while, after an iron induced remission had been produced, the serum iron increases were large. In contrast to these results are those recently reported by Hahn and his associates (26). These latter investigators fed ferric chloride or ferric sulphate containing iron in a radioactive form to normal dogs and to dogs with an iron deficiency. They concluded from increases in serum, organ, and whole blood iron that "iron is absorbed only in traces by the non-anemic dog but in abundance by the anemic dog depleted of its iron" (26, page 2285).

We have studied several individuals in a similar manner and have obtained divergent results. The serum iron increases which followed the oral administration of ferrous sulphate were determined in these patients with hypochromic, microcytic anemias both before and after therapy. One type of response is illustrated by the data obtained on G W, an adult male with normal gastric acidity (Figure 9). When the iron deficiency was marked, an increase in serum iron from 0.02 mgm per cent to approximately 0.30 mgm per cent occurred, after a prolonged period of iron medication, the rise was only from 0.103 mgm per cent to 0.213 mgm per cent. This finding is in accord with that noted by Hahn and his coworkers. However, there was an occasional patient who showed similar serum iron increases throughout the whole course of the disease. One of these individuals was a young girl who had developed her state of iron deficiency as a result of excessive uterine bleeding secondary to essential thrombocytopenic purpura (Figure 20). Her uterine bleeding was successfully controlled during the period of study by the use of "antutrin-S". Iron was given in therapeutic amounts and the hypochromia of her red blood cells corrected before splenectomy was performed. Ab-

^a Obtained from Frederick Stearns and Company, Detroit.



Serum Iron Responses to 5 mg. Fe⁺⁺ (as Ferrous Sulfate) per Kilogram Body Weight.

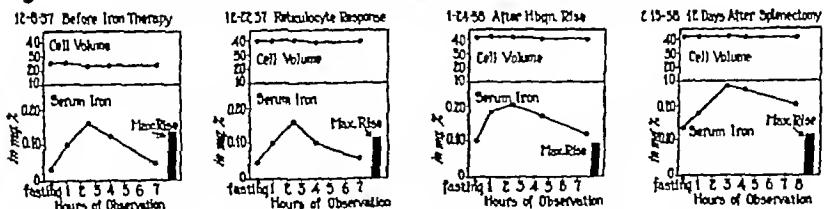


FIG. 20. RESPONSE OF A PATIENT WITH THROMBOCYTOPENIC PURPURA AND HYPOCHROMIC MICROCYTIC ANEMIA TO IRON THERAPY AND TO SPLENECTOMY

sorption was studied by serum iron curves (1) before therapy (2) one week after iron medication was begun (3) after the mean corpuscular hemoglobin concentration of the erythrocytes had returned to the normal range, and (4) near the end of the convalescent period following splenectomy. The increases above the basal level

were essentially the same at all 4 times. It is difficult to correlate this type of result with the selective absorption hypothesis unless it is assumed that, during the iron deficiency periods of study, the iron absorbed into the serum is removed at an abnormally rapid rate by the iron hungry organs of storage and utilization.

In the second paper of this series (2, page 630), it was pointed out that 3 patients had failed to show serum iron increases following orally administered amounts of iron that were considerably in excess of the quantities used in the present study "All three of these patients were acutely ill at the time the observations were made. Any reason otherwise for their failure to respond

is not at present apparent" A re-analysis of the data from these 3 individuals reveals that the first 2 had myelophthasic anemias with marked hypoplasia of the erythrocytogenic elements in the bone marrow, the third had pernicious anemia. In all 3 the basal serum iron values were high, and in all 3 the iron storage depots were probably well supplied. This suggested that perhaps we were dealing in these instances with other examples of selective iron absorption, and that the serum iron values failed to show an increase because the intestinal mucosa was functioning by rejecting the iron. In order to test this possibility further, serum iron absorption curves were followed in 5 other patients with addisonian pernicious anemia both before therapy and after liver induced remissions. Either ferrous chloride or ferrous sulphate was selected for administration. The data presented in Figure 21 are

illustrative of the findings obtained in all cases: no serum iron increases occurred during the relapse phases of the disease, but after the peripheral blood level of erythrocytes and hemoglobin had been raised to normal with liver extract, striking increases were noted. On one of the patients, N J T, a 49-year-old white male, 3 sets of observations were made (Figure 22) (1) before therapy, (2) at the height of the reticulocyte response to liver medication, and (3) after remission was complete. It is interesting to observe that at the time of the reticulocyte response the serum iron curve rose practically as much as at the time of the final period. A similar study was made on Patient R R, a white woman in late middle life who had the classical manifestations of the disease (Figure 23). This patient is of particular interest since the reticulocytosis which occurred was induced by the ingestion of large amounts of unautolyzed yeast, a regime that was undertaken to confirm the report of Wintrobe (59). The second observation on this patient was made at the time of the upswing in the reticulocyte curve, the serum iron rise was definite, but less than that which occurred several days later after the peak of the reticulocytosis. Two subsequent increases after further therapy agreed

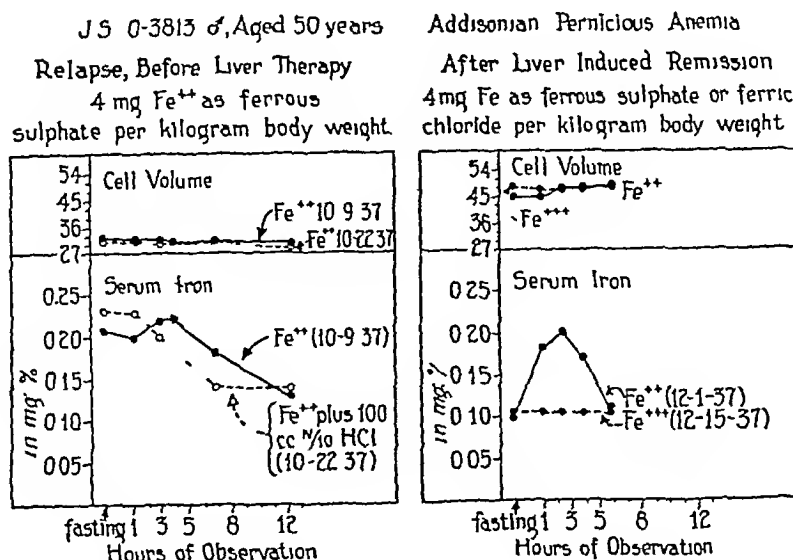


FIG 21 SERUM IRON RESPONSES TO ORAL DOSES OF IRON SALTS BEFORE AND AFTER LIVER THERAPY IN A PATIENT WITH ADDISONIAN PERNICIOUS ANEMIA

Iron administered in each instance immediately after fasting specimen.

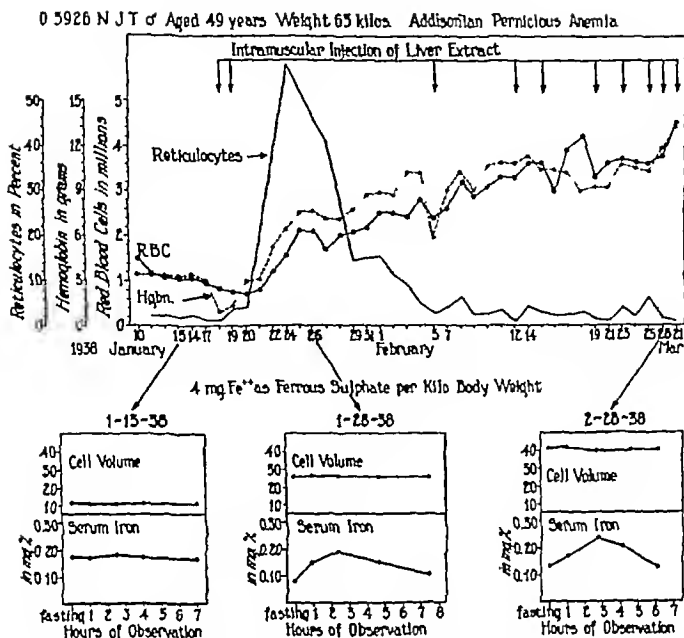


FIG. 22. SERUM IRON INCREASES FOLLOWING ORAL ADMINISTRATION OF FERROUS SULPHATE IN A PATIENT WITH ADDISONIAN PERNICIOUS ANEMIA BEFORE AND AFTER LIVER THERAPY

well in order of magnitude with that observed at the height of the reticulocyte response. These findings are not too readily explained on the basis of selective intestinal absorption of iron. At the time of the reticulocyte response the storehouses in these patients must undoubtedly have had an adequate supply of iron still retained. However, because of the increased synthesis of hemoglobin in the bone marrow (2) the fasting serum iron level had returned to normal or below normal values. It may be that that change in the physiological equilibrium was sufficient in itself to stimulate the intestinal mucosa to begin again its process of assimilating iron. It is interesting to note in this connection that the height to which the serum iron values rose in the absorptive phase after liver therapy was rarely higher than the fasting serum iron level had been during relapse.

A serious objection to this explanation arose

when it was observed that several patients with advanced carcinomatosis and one during the terminal phases of chronic glomerular nephritis failed to develop an increase in serum iron following ferrous sulphate in spite of the fact that their initial levels were normal or subnormal. It may be, therefore, that this absorptive difficulty is common to all patients who are critically ill at least no absorptive increases in serum iron have ever been demonstrated in critically ill individuals. Groen (60), in addition, has reported that glucose is absorbed less readily by patients with pernicious anemia while in relapse than when in remission. The change in absorption produced by liver extract may, therefore, be a general rather than a specific one.

Two pertinent observations have been made (1) at least some patients with iron deficiencies have greater proportionate serum iron increases

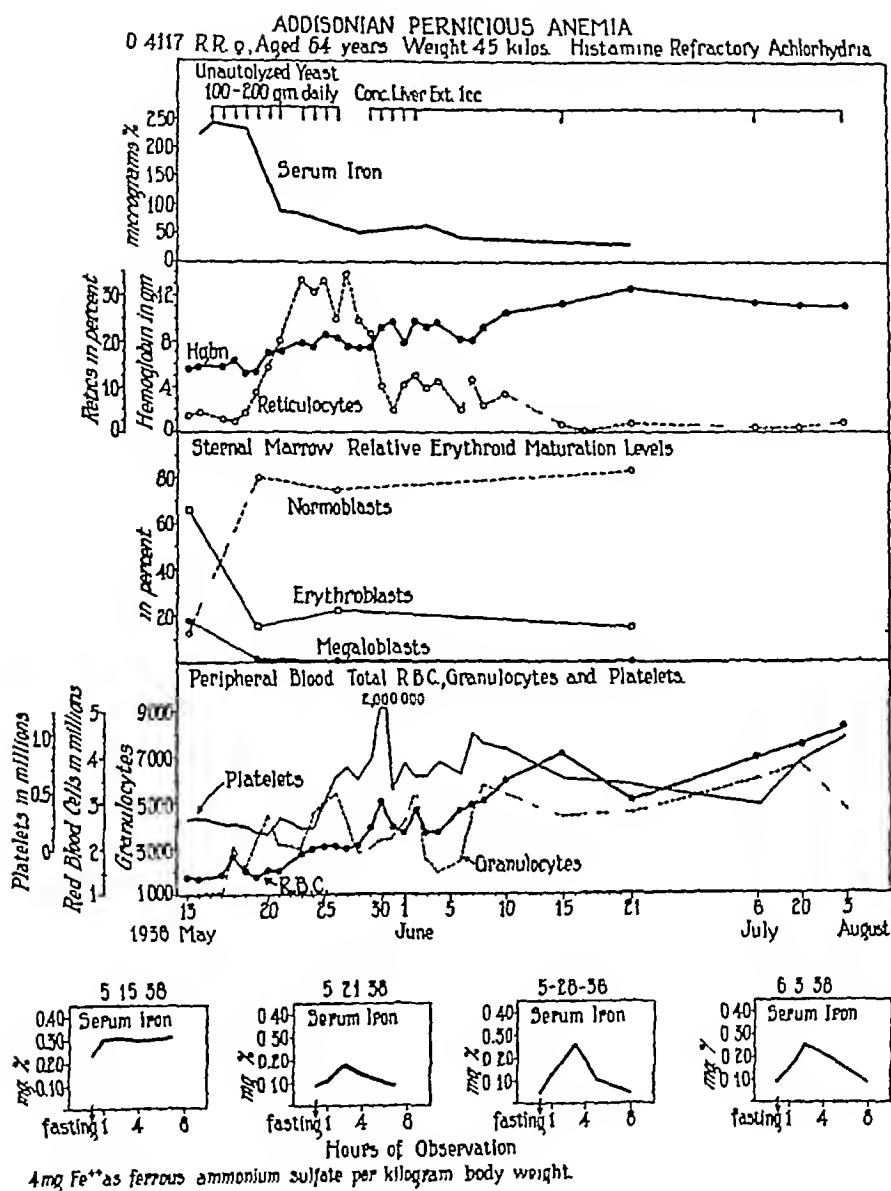


FIG 23 SERUM IRON INCREASES FOLLOWING ORAL ADMINISTRATION OF FERROUS AMMONIUM SULPHATE IN A PATIENT WITH ADDISONIAN PERNICIOUS ANEMIA BEFORE AND AFTER THERAPY (MASSIVE DOSES OF UNAUTOLYZED BREWER'S YEAST)

to the same amount of soluble iron salts than do normal individuals, and (2) patients with pernicious anemia in relapse fail to show any response of the serum iron fraction to orally administered iron salts during relapse but have good increases after specific therapy has been instituted. These facts are compatible with the concept of selective intestinal absorption of iron, but offer no conclusive proof that the intestine actually possesses that ability.

SUMMARY AND CONCLUSIONS

Comprehensive reviews of iron metabolism which include an analysis of the investigative approach to the problem of iron absorption have been made recently by Robschert-Robbins (61), McCance and Widdowson (19), Hahn (18), and Heath and Patek (11). The present investigation directs attention to the fact that, since serum iron has been demonstrated to be transport iron (2, 4, 28b), a new objective approach to the study

of iron resorption from the gastro intestinal tract is available in the measurement of serum (transport) iron increases following the oral administration of iron in various forms. It has been emphasized that serum iron is influenced by a number of physiological factors some of which affect its removal from the blood stream. The method therefore, does not measure the total amount of absorption but rather the fact and the degree of absorption. It is applicable to the comparative study of serum iron responses in the same individual to different forms of iron under varying gastro-intestinal conditions, and in contrasting states of hematopoietic activity. With its use we have demonstrated that

1 Absorption of iron from the gastro-intestinal tract was reflected directly by an increase in serum iron and not through an intermediate rise in the iron of the thoracic duct lymph

2 The disappearance of intravenously injected soluble iron salts from the blood stream occurred slowly during a period of hours

3 The height of the serum iron increase following the ingestion of iron was directly proportional to the quantity given up to that point at which intestinal irritation was great enough to interfere materially with intestinal motility

4 When identical amounts of ferrous sulphate were given to any subject in hematologic equilibrium, the serum iron curves produced closely paralleled each other

5 Water soluble, highly ionized ferrous salts produced comparable increases in serum iron values when given in proportionate amounts to patients with either an achlorhydria or normal gastric acidity. These findings suggest that the ease of ionization of ferrous salts, under those conditions of acidity or alkalinity present in the gastro-intestinal tract, is the important factor in determining their relative rates of absorption. The nature of the anion, except as it influences this factor, would seem to be relatively unimportant

6. In the majority of instances, the serum iron absorption curves were higher following the ingestion of ferrous than of ferric salts. When ferric salts were given to subjects along with relatively large amounts of either ascorbic acid or sodium formaldehyde sulfoxylate, the serum

iron responses were uniformly as good as those produced by ferrous salts of the same anion. These two reducing agents were likewise frequently capable of increasing the rise obtained following the ingestion of ferrous preparations. These findings are presented as additional evidence for the conviction that iron is absorbed largely, if not entirely, in the ferrous form

7 Differences in gastric acidity exerted no measurable effect on the serum iron absorption curves following the oral administration of ferrous or ferric salts. It is suggested, however that such large amounts of the various iron salts were used that their own acidity was sufficiently great to overcome the effect of naturally occurring hypochlorhydria in delaying the formation of insoluble and undissociated iron compounds which may occur above pH 5

8 No increases in serum iron were obtained following administration of the insoluble ferrous and ferric phosphate.

9 When subjects were permitted to take food just prior to, or immediately following the ingestion of iron salts there was observed frequently, but not constantly, a smaller rise in the serum iron than occurred under fasting conditions

10 Bile pigment, chlorophyll and its derivatives the secondary anemia liver fraction, and gastric mucin, under the conditions of these experiments, failed to alter the height or character of the serum iron absorption curves produced by ferrous sulphate.

11 Patients with an iron deficiency anemia frequently showed evidence of greater absorption of iron before therapy than after they had been adequately treated. On the other hand, patients with Addisonian pernicious anemia in relapse showed no demonstrable increase in their already higher than normal serum iron values when ferrous salts were given orally, following a liver induced remission, however, normal absorption curves were readily obtained. These observations are compatible with the hypothesis that the intestinal mucosa may, under certain circumstances and within certain limits, assimilate or reject iron according to the body's need. They do not however constitute conclusive evidence for this hypothesis

From the data available in the literature and

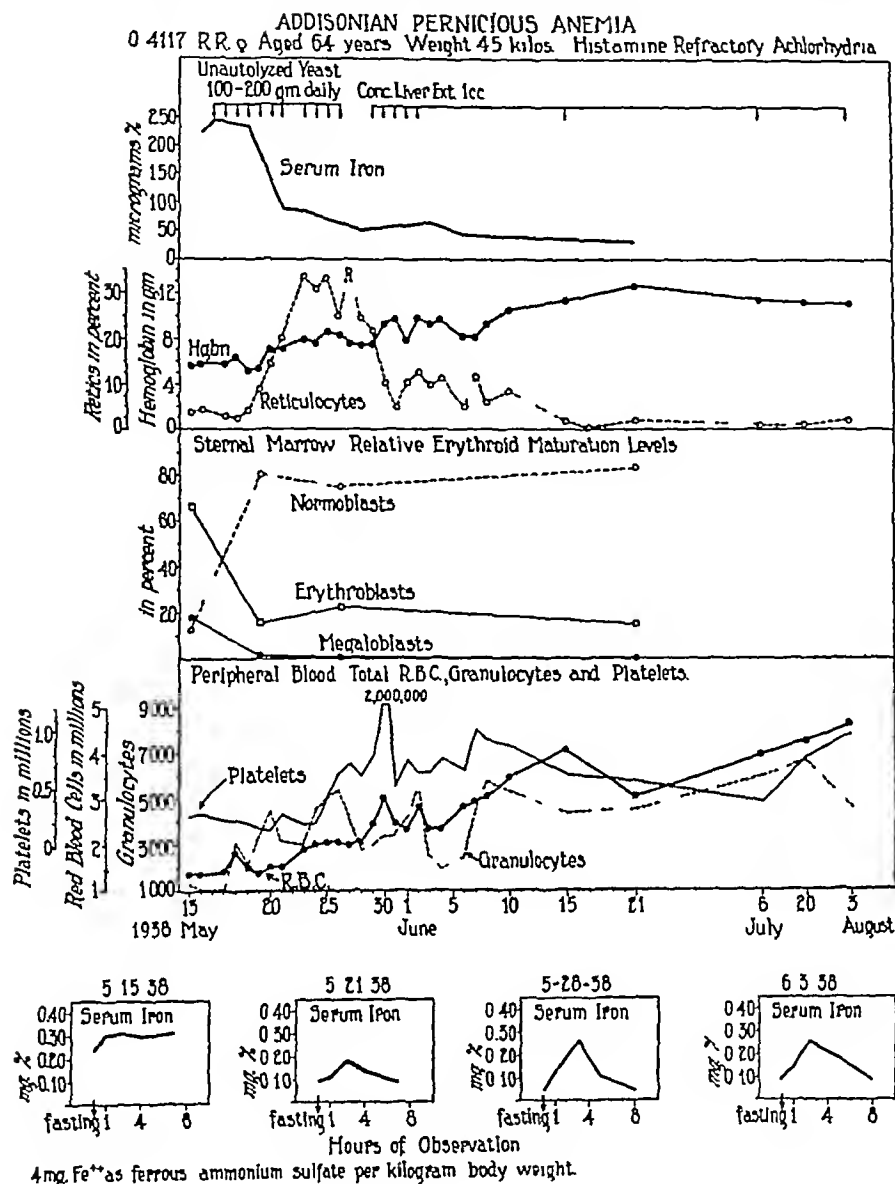


FIG 23 SERUM IRON INCREASES FOLLOWING ORAL ADMINISTRATION OF FERROUS AMMONIUM SULPHATE IN A PATIENT WITH ADDISONIAN PERNICIOUS ANEMIA BEFORE AND AFTER THERAPY (MASSIVE DOSES OF UNAUTOLYZED BREWER'S YEAST)

to the same amount of soluble iron salts than do normal individuals, and (2) patients with pernicious anemia in relapse fail to show any response of the serum iron fraction to orally administered iron salts during relapse but have good increases after specific therapy has been instituted. These facts are compatible with the concept of selective intestinal absorption of iron, but offer no conclusive proof that the intestine actually possesses that ability.

SUMMARY AND CONCLUSIONS

Comprehensive reviews of iron metabolism which include an analysis of the investigative approach to the problem of iron absorption have been made recently by Robschert-Robbins (61), McCance and Widdowson (19), Hahn (18), and Heath and Patek (11). The present investigation directs attention to the fact that, since serum iron has been demonstrated to be transport iron (2, 4, 28b), a new objective approach to the study

- 23a. Fowler W M., Barer A. P., and Spielhagen, G. F., Retention and utilization of small amounts of orally administered iron. *Arch. Int. Med.*, 1937, 59 1024
24. Barkan G., and Schaale O.,
 - (a) Chemischer Aufbau und physiologische Bedeutung des "leicht abspaltbaren" Bluteisens. 13. Mitteilung in der Reihe der Eisenstudien. *Ztschr f. physiol. Chem.*, 1937 248 96.
 - (b) Bildungsmöglichkeiten und Eigenschaften der Pseudohämoglobine. 14. Mitteilung in der Reihe der Eisenstudien. *Ztschr f. Physiol. Chem.*, 1938, 253 83
25. Cortis-Jones, B., and Lemberg R., The chemical mechanism of hemoglobin breakdown. *Proc. of the 16th International Physiological Conference, Kongressbericht II* p 251
26. Hahn, P. F., Bale, W. F., Lawrence, E. O., and Whipple, G. H., Radioactive iron and its metabolism in anemia. *J. A. M. A.* 1938 111 2285
27. Thoenes, F., and Aschaffenburg, R., Der Eisenstoffwechsel des Wachsenen Organismus. Abhandlung aus d. kinderheilkunde und ihren Grenzgebieten, Karger Berlin 1934
28. Hellmeyer Ludwig and Piltner Kurt,
 - (a) Eisenmangelzustände und ihre Behandlung *Klin. Woch.*, 1936, 15, 1669
 - (b) Das Serum Eisen und die Eisenmangelkrankheit (Pathogenese, symptomologie und therapie) 1937 Gustav Fischer Jena.
29. Gaule, J., Der Nachweis des Resorbierten Eisens in der Lymphe des Ductus thoracicus. *Deutsche med. Wochenschr.*, 1896 22 373.
30. Muller F., Beiträge zur Frage nach der Wirkung des Eisens bei Experimentell Erzeugter Anämie. *Arch. f. Path. Anat.*, 1901 164 436
31. Höber R., Ueber Resorption im Darm. *Arch. f. d. ges. Physiol.*, 1903 94 337
32. Lintzel, W., Neuere Ergebnisse der Erforschung des Eisenstoffwechsels. *Ergebn. d. Physiol* 1931 31 844
33. Fontès, G., and Thivolle, L., Bilan du Fer chez le Chien rendu anémique par Saignées répétées. *Compt. Rend. Soc. de Biol.*, 1932, 109 911
34. Starkenstein, E., and Weden, H.,
 - (a) Über das anorganische Eisen des Organismus. *Arch. f. Exper. Path. u. Pharmacol.*, 1928 134, 274
 - (b) Über das Schicksal des Eisens im Organismus nach Zufuhr von complexen Verbindungen mit anorganisch und organisch gebundenem Eisen. *Ibid.*, 1930 150 354
35. Barkan, G.,
 - (a) Eisenstudien, Die Verteilung des leicht abspaltbaren Eisens zwischen Blutkörperchen und Plasma und sein Verhalten unter Experimentellen Bedingungen. *Ztschr f. Physiol. Chem.*, 1927 171 194.
 - (b) Über das Verhalten von anorganischem Eisen nach Zusatz zum Blute. 7. Mitteilung in der Reihe der Eisenstudien. *Ibid.*, 1933, 216, 17
36. Polson, C. J.,
 - (a) Fate of colloidal iron administered intravenously *J. Path. and Bact.*, 1928, 31 445
 - (b) Fate of colloidal iron administered intravenously, long experiments. *Ibid.*, 1929 32, 247
37. Hahn, P. F., and Whipple, G. H., Iron metabolism its absorption, storage and utilization in experimental anemia. *Am. J. M. Sc.* 1936, 191, 24
38. Starkenstein, E., and Harvalik, Z., Über eine im intermedären Eisenstoffwechsel entstehende Ferri globulinverbindung. *Arch. f. exper. Path. u. Pharmacol.*, 1933 172 75
39. Starkenstein, E., Über die Resorbierbarkeit von Eisenverbindungen aus dem Verdauungskanal. *Arch. f. exper. Path. u. Pharmacol.* 1927 127 101
40. Wallbach, G., Die Eisenresorption als Voraussetzung der Anämiebehandlung. Weitere Untersuchungen über die verschiedenen Resorptionserscheinungen der einzelnen Eisenpräparate. *Folia haemat.*, 1936, 54, 201
41. Starkenstein, E. *Handbuch der allgemeinen Hämatologie*, Band II 2. [Hirschfeld, H., and Hittmair, A. eds.] Berlin, Urban, 1934 p. 1384
42. Reimann, F., and Fritsch, F., Vergleichende Untersuchungen zur therapeutischen Wirksamkeit der Eisenverbindungen bei den sekundären Anämien. *Ztschr f. klin. Med.*, 1930 115 13
43. Hellmeyer L., Personal communication.
44. Robinson, C. S., The hydrogen ion concentration of the contents of the small intestine. *J. Biol. Chem.*, 1937 108, 403.
45. Halvorsen, H., and Starkey R., Studies on the transformation of iron in nature. I Theoretical considerations. *J. Phys. Chem.*, 1927 31, 626.
46. Smythe, C. V., and Schmidt, C. L. A., Studies on the mode of combination of iron with certain proteins amino acids and related compounds. *J. Biol. Chem.*, 1930 88, 241
47. Tompsett, S. L.,
 - (a) Studies of the complexes of iron with various biological materials. *Biochem. J.*, 1934 28, 1802.
 - (b) The copper and "inorganic" iron contents of human tissues. *Biochem. J.*, 1935 29 480.
48. Kellogg, F., and Mettler S. R., Effect of alkaline therapy for peptic ulcer on utilization of dietary iron in regeneration of hemoglobin. *Arch. Int. Med.*, 1936, 58, 278.
49. Strauss, Maurice B., Role of the gastro-intestinal tract in conditioning deficiency disease. *J. A. M. A.*, 1934 103, 1
50. Barer A. P., and Fowler W M., Influence of gastric acidity and degree of anemia on iron retention. *Arch. Int. Med.*, 1937 59 785
51. Mettler S. R., and Minot, G. R., Effect of iron on blood formation as influenced by changing acidity of gastroduodenal contents in certain cases of anemia. *Am. J. M. Sc.*, 1931 181 25
52. Minot, G. R., and Heath, C. W., Response of reticulocytes to iron. *Am. J. M. Sc.*, 1932, 183 110.

that which this communication presents, the following picture of iron absorption may tentatively be constructed. When ingested iron reaches the stomach, it is subjected to the influences of the prevailing acidity. The free hydrochloric acid normally present apparently has two functions (1) to ionize and dissolve iron not already present in solution nor in an ionized state, and (2) to delay the formation of insoluble and undissociated iron compounds. Since these form at a pH above 5.0, the change to them would tend to occur to some degree, at least, in the stomachs of patients with achlorhydria. When the iron is delivered to the duodenum, it is subjected to two influences: the alkaline intestinal juices and certain reducing agents. The latter tend to reduce any trivalent iron to the ferrous form before the change to non-ionizable salts has occurred. Iron is absorbed from the intestinal tract largely, if not entirely, as ferrous iron. The degree to which ferric salts are assimilated would seem to depend upon the capacity of the intestinal contents to reduce them. It is the consensus of opinion that absorption takes place largely in the upper portion of the small intestine. When iron is absorbed, it passes directly into the blood plasma and is not to any extent collected by the intestinal lymph channels. As more data are accumulated, this working hypothesis based on information now available will undoubtedly be altered and enlarged.

BIBLIOGRAPHY

- 1 Moore, Carl V., Arrowsmith, Wm. R., Quilligan, J. J., Jr., and Read, J. T., Studies in iron transportation and metabolism. I. Chemical methods and normal values for plasma iron and "easily split-off" blood iron. *J. Clin. Invest.*, 1937, 16, 613
- 2 Moore, Carl V., Doan, Charles A., and Arrowsmith, Wm. R., Studies in iron transportation and metabolism. II. Mechanism of iron transportation: its significance in iron utilization in anemic states of varied etiology. *J. Clin. Invest.*, 1937, 16, 627
- 3 Moore, Carl V., Minnich, Virginia, and Welch, Jo, Studies in iron transportation and metabolism. III. The normal fluctuations of serum and "easily split-off" blood iron in individual subjects. *J. Clin. Invest.*, 1939, 18, 543
- 4 Moore, Carl V., and Doan, Charles A., Correlation of serum iron, bone marrow and blood cell changes following specific therapy in the macrocytic anemias. *Arch. Path. (Proc.)*, 1937, 23, 738
- 5 Heubner, W.,
(a) Bemerkung zur Eisentherapie. *Ztschr. f. Klin. Med.*, 1924, 100, 675
(b) Über "Organische" Eisenpräparate. *Klin. Wchnschr.*, 1926, 5, 588
- 6 Posener, K., Ueber die theoretischen Grundlagen der Eisentherapie. *Therap. d. Gegenw.*, 1927, 68, 541
- 7 Wiechowski, W., Die Eisentherapie im Lichte der neueren Forschung. *Med. Klin.*, 1927, 23, 1765
- 8 Starkenstein, E., Die Derzeitigen Pharmakologischen Grundlagen einer Rationellen Eisentherapie. *Klin. Wchnschr.*, 1928, 7, 217
- 9 Fullerton, H. W., Treatment of hypochromic anaemia with soluble ferrous salts. *Edinburgh M. J.*, 1934, 41, 99
- 10 Wits, L. J., Therapeutic action of iron. *Lancet*, 1936, 1, 1
- 11 Heath, Clark W., and Patek, Arthur J., Jr., Anemia of iron deficiency. *Medicine*, 1937, 16, 267
- 12 Fowler, W. M., and Barer, A. P., Iron retention following use of ferric ammonium citrate in hypochromic anemia. *J. A. M. A. (Proc.)*, 1935, 104, 144
- 13 Widdowson, E. M., and McCance, R. A., Absorption and excretion of iron before, during and after period of very high intake. *Biochem. J.*, 1937, 31, 2029
- 14 Abbott, W. O., and Miller, T. G., Intubation studies of human small intestine, technic for collection of pure intestinal secretion and for study of intestinal absorption. *J. A. M. A.*, 1936, 106, 16
- 15 Whipple, G. H., and Rabschtein-Robbins, F. S., Iron and its utilization in experimental anemia. *Am. J. M. Sc.*, 1936, 191, 11
- 16 Heath, C. W., Strauss, M. B., and Castle, W. B., Quantitative aspects of iron deficiency in hypochromic anemia, parenteral administration of iron. *J. Clin. Invest.*, 1932, 11, 1293
- 17 Heath, C. W., Oral administration of iron in hypochromic anemia. *Arch. Int. Med.*, 1933, 51, 459
- 18 Hahn, P. F., Metabolism of iron. *Medicine*, 1937, 16, 249
- 19 McCance, R. A., and Widdowson, E. M.,
(a) Absorption and excretion of iron. *Lancet*, 1937, 2, 680
(b) Absorption and excretion of iron following oral and intravenous administration. *J. Physiol.*, 1938, 94, 148
- 20 Groen, J., and Taylor, F. H. L., Absorption of iron compounds from upper part of small intestine. *Proc. Soc. Exper. Biol. and Med.*, 1937, 36, 694
- 21 Brock, J. F., Relation between hypochromic anaemias and iron deficiency. *Brit. M. J.*, 1937, 1, 314
- 22 Brock, J. F., and Hunter, D., Fate of large doses of iron administered by mouth. *Quart. J. Med.*, 1937, 6, 5
- 23 Fowler, W. M., and Barer, A. P., Retention and utilization of orally administered iron. *Arch. Int. Med.*, 1937, 59, 561

- 23a. Fowler, W. M., Barer A. P., and Spielhagen, G. F., Retention and utilization of small amounts of orally administered iron. *Arch. Int. Med.*, 1937 59 1024
- 24 Barkan G., and Schaale, O.,
 - (a) Chemischer Aufbau und physiologische Bedeutung des "leicht abspaltbaren" Blutesens. 13. Mitteilung in der Reihe der Eisenstudien. *Ztschr. f. physiol. Chem.*, 1937, 248, 96.
 - (b) Bildungsmöglichkeiten und Eigenschaften der Pseudohämoglobine. 14. Mitteilung in der Reihe der Eisenstudien. *Ztschr. f. Physiol. Chem.*, 1938, 253, 83
- 25 Cortis Jones, B., and Lemberg R., The chemical mechanism of hemoglobin breakdown. *Proc. of the 16th International Physiological Conference, Kongressbericht II* p 251
26. Hahn, P. F., Bale, W. F., Lawrence, E. O., and Whipple, G. H., Radioactive iron and its metabolism in anemia. *J. A. M. A.*, 1938, 111 2285.
- 27 Thoenes, F., and Aschaffenburg R., Der Eisenstoffwechsel des Wachsenden Organismus. Abhandlung aus d. Kinderheilkunde und ihren Grenzgebieten, Karger Berlin 1934
28. Heilmeyer Ludwig and Plotner Kurt,
 - (a) Eisenmangelzustände und ihre Behandlung *Klin. Woch.*, 1936 15 1669
 - (b) Das Serum-eisen und die Eisenmangelkrankheit (Pathogenese, symptomologie und therapie) 1937 Gustav Fischer Jena.
- 29 Gaule, J., Der Nachweis des Resorbierten Eisens in der Lymphe des Ductus thoracicus. *Deutsche med. Wochenschr.*, 1896, 22 373
- 30 Müller F., Beiträge zur Frage nach der Wirkung des Eisens bei Experimentell Erzeugter Anämie. *Arch. f. Path. Anat.*, 1901 164 436.
- 31 Höber R., Ueber Resorption im Darm. *Arch. f. d. ges. Physiol.*, 1903 94 337
32. Lintzel, W., Neuere Ergebnisse der Erforschung des Eisenstoffwechsels. *Ergebn. d. Physiol.*, 1931 31 844
33. Fontès G., and Thivolle, L., Bilan du Fer chez le Chien rendu anémique par Saignées répétées *Compt. Rend. Soc. de Biol.*, 1932, 109 911
- 34 Starkenstein, E., and Weden, H.,
 - (a) Über das anorganische Eisen des Organismus. *Arch. f. Exper. Path. u. Pharmacol.*, 1928, 134 274
 - (b) Über das Schicksal des Eisens im Organismus nach Zufuhr von komplexen Verbindungen mit anorganisch und organisch gebundenem Eisen. *Ibid.*, 1930, 150 354
- 35 Barkan, G.,
 - (a) Eisenstudien, Die Verteilung des leicht abspaltbaren Eisens zwischen Blutkörperchen und Plasma und sein Verhalten unter Experimentellen Bedingungen. *Ztschr. f. Physiol. Chem.*, 1927 171, 194
 - (b) Über das Verhalten von anorganischem Eisen nach Zusatz zum Blute. 7. Mitteilung in der Reihe der Eisenstudien. *Ibid.*, 1933 216, 17
- 36 Polson, C. J.,
 - (a) Fate of colloidal iron administered intravenously *J. Path. and Bact.*, 1928, 31, 445.
 - (b) Fate of colloidal iron administered intravenously long experiments. *Ibid.*, 1929 32 247
- 37 Hahn, P. F., and Whipple G. H. Iron metabolism its absorption, storage and utilization in experimental anemia. *Am. J. M. Sc.*, 1936, 191 24
38. Starkenstein, E., and Harvalik, Z., Über eine im intermediären Eisenstoffwechsel entstehende Ferri globulinverbindung *Arch. f. exper. Path. u. Pharmacol.*, 1933 172, 75
- 39 Starkenstein, E., Über die Resorbierbarkeit von Eisenverbindungen aus dem Verdauungskanal. *Arch. f. exper. Path. u. Pharmacol.*, 1927 127 101
- 40 Wallbach, G., Die Eisenresorption als Voraussetzung der Anämiebehandlung Weitere Untersuchungen über die verschiedenen Resorptionsercheinungen der eisenreichen Eisenpräparate. *Folia haemat.*, 1936 54, 201
- 41 Starkenstein, E., Handbuch der allgemeinen Hämatalogie, Band II, 2. (Hirschfeld, H., and Hittmair A., eds.) Berlin, Urban, 1934 p. 1384
42. Reimann, F. and Fritsch, F., Vergleichende Untersuchungen zur therapeutischen Wirksamkeit der Eisenverbindungen bei den sekundären Anämien. *Ztschr. f. klin. Med.*, 1930 115, 13
- 43 Heilmeyer L., Personal communication.
- 44 Robinson, C. S. The hydrogen ion concentration of the contents of the small intestine. *J. Biol. Chem.*, 1937 108, 403
45. Halvorsen, H., and Starkey R., Studies on the trans formation of iron in nature. I. Theoretical considerations. *J. Phys. Chem.*, 1927 31 626.
46. Smythe, C. V., and Schmidt, C. L. A., Studies on the mode of combination of iron with certain proteins, amino acids and related compounds. *J. Biol. Chem.*, 1930 88, 241
- 47 Tompsett, S. L.,
 - (a) Studies of the complexes of iron with various biological materials. *Biochem. J.*, 1934 28, 1802.
 - (b) The copper and "inorganic" iron contents of human tissues. *Biochem. J.*, 1935 29 480
48. Kellogg F., and Mettler S. R., Effect of alkaline therapy for peptic ulcer on utilization of dietary iron in regeneration of hemoglobin. *Arch. Int. Med.* 1936, 58, 278.
- 49 Strauss Maurice B., Rôle of the gastro-intestinal tract in conditioning deficiency disease. *J. A. M. A.*, 1934 103 1
50. Barer A. P., and Fowler W. M., Influence of gastric acidity and degree of anemia on iron retention. *Arch. Int. Med.*, 1937 59 785
- 51 Mettler S. R., and Minot, G. R., Effect of iron on blood formation as influenced by changing acidity of gastroduodenal contents in certain cases of anemia. *Am. J. M. Sc.*, 1931 181 25
52. Minot, G. R., and Heath, C. W., Response of reticulocytes to iron. *Am. J. M. Sc.*, 1932, 183, 110.

cose intravenously and 15 units of insulin subcutaneously

The blood samples were analyzed by Abel's (15) macro-method. In our laboratory the recoveries of known solutions of alcohol showed an average error of ± 5.1 per cent. The differences between duplicate analyses of the same blood samples in these experiments never exceeded 10 per cent.

RESULTS

The data are presented in Tables I and II. Table I compares the observations on the untreated controls and the group injected with 15 units of insulin subcutaneously. It may be seen that the average amount of alcohol which disappeared from the blood in 2 hours was independent of the original blood concentration. In the moderately intoxicated group it was 48 ± 2.3 mgm per cent, while the severely intoxicated group showed an average reduction of 54 ± 2.0 mgm per cent. This difference was not significant.

TABLE I

Observations on untreated patients, and those injected with 15 units of insulin subcutaneously (milligrams per cent of alcohol)

Control observations				Patients injected with 15 units of insulin			
Blood concentration	Change in 2 hours	Blood concentration	Change in 2 hours	Blood concentration	Change in 2 hours	Blood concentration	Change in 2 hours
275	45	600	75	280	102	575	50
275	69	550	50	250	61	525	50
275	75	525	50	250	67	525	50
275	50	500	50	250	50	525	75
274	60	400	53	200	40	425	50
237	50	347	52	175	25	425	50
230	54	325	51	125	25	325	50
225	75	325	50			300	86
206	59					300	20
200	66						
200	50						
200	34						
187	24						
166	46						
150	37						
150	42						
125	35						
90	35						
Average	48 ± 2.3		54 ± 2.0		53 ± 6.7		53 ± 3.8
Difference from control					5 ± 7.1		1 ± 4.3

TABLE II

Observations on patients treated with 50 cc of 50 per cent glucose, and with glucose plus 15 units of insulin (milligrams per cent of alcohol)

50 cc of 50 per cent glucose				Glucose plus insulin			
Blood concentration	Change in 2 hours	Blood concentration	Change in 2 hours	Blood concentration	Change in 2 hours	Blood concentration	Change in 2 hours
280	26	600	100	275	100	600	200
275	25	525	75	250	175	600	275
254	66	525	125	250	87	550	225
250	38	500	175	250	88	525	125
250	50	450	135	200	91	500	200
250	50	400	100	180	64	450	150
250	50	350	100	175	50	425	150
225	50	325	115	150	57	425	232
212	45	315	125	105	80	380	205
200	75	300	50			375	125
200	55					350	125
175	60					330	80
						320	195
						300	140
						300	75
Average	49 ± 3.0		110 ± 7.4		86 ± 7.4		167 ± 9.7
Difference from control	1 ± 3.7		56 ± 7.6		38 ± 7.8		113 ± 9.7

Therapy with 15 units of insulin had no effect on the disappearance of alcohol from the blood. Both groups had an average fall of blood alcohol of 53 mgm per cent and statistical analysis revealed that the differences from the controls were not significant.

Table II is a summary of the patients injected with 50 cc of 50 per cent glucose intravenously, and those injected with the glucose plus 15 units of insulin. Patients whose blood alcohol concentration was less than 300 mgm per cent showed an average reduction of 49 ± 3.0 mgm per cent after treatment with glucose. This was not significantly different from the control group. The more severely intoxicated group treated with glucose had an average reduction of blood alcohol of 110 ± 7.4 mgm per cent, a statistically significant increase over the control group. Treatment with glucose and insulin resulted in a greater fall in blood alcohol in both the moderately intoxicated as well as the severely intoxicated patients. The average reduction was 86 ± 7.4 mgm per cent and 167 ± 9.7 mgm per cent in the 2 groups respectively.

The clinical appearance of the patients treated with the various types of therapy presented marked differences. Patients who were injected

with 15 units of insulin alone invariably showed slight sweating and a fall in temperature. Those who were mentally clear frequently complained of feelings of hunger about 2 to 3 hours after the injection. No significant changes were noted in the group treated with glucose alone. The patients treated with glucose and insulin recovered consciousness between $1\frac{1}{2}$ to 2 hours after treatment and in some cases showed adequate motor coordination within 4 to 5 hours. In most of these cases a rational history could be obtained at the end of 2 hours.

DISCUSSION

The oxidation of alcohol is of interest both from the purely physiological as well as the clinical points of view. Most investigators have found that the oxidation of ingested alcohol is accelerated when food, especially carbohydrate is simultaneously oxidized. In a previous publication we have shown that the oxidation of alcohol *in vitro* is in some way accelerated when glucose was oxidized in the same solution. This analogy, although suggestive, cannot be taken as proof since the oxidative processes involved in no way resemble those in the animal organism.

We found that the administration of glucose alone had a moderate effect on the rate of disappearance of alcohol in the severely intoxicated patients while the moderately intoxicated group showed no change from the controls. It was suggested that the alcoholic patients were in various states of undernutrition and could not oxidize the glucose given. This seemed probable in view of the fact that Traugott (16) found that as little as a 3-day fast produced a marked diminution of sugar tolerance in humans. We have examined the sugar tolerance in a group of patients similar to those reported in this paper. In most cases there was an abnormal elevation of the tolerance curve, with a return to normal when they were tested one week later. During the week the patients were maintained on a normal hospital diet (17). It is therefore likely that the glucose alone did not accelerate the oxidation of alcohol in most cases because the glucose was not oxidized. The addition of insulin assures the oxidation of the glucose and the oxidation of the alcohol in the body is thus accelerated.

The prognosis of acute alcoholism is ordinarily very good for recovery and patients usually receive very little active therapy. However, fatalities have been reported, and any patient who reaches the stage of alcoholic coma may be considered dangerously ill. Therapy should be aimed at a reduction of the alcohol content of the body. Robinson and Selesnick (4) increased the excretion through the lungs by increasing the respiratory volume with 10 per cent carbon dioxide in oxygen. They were able to produce a reduction of blood alcohol ranging from 8 to 137 mgm. per cent in $\frac{1}{2}$ hour in severely intoxicated patients. In the present experiments the administration of glucose and insulin caused a reduction of blood alcohol ranging from 75 to 275 mgm. per cent in 2 hours in a comparable group of patients. Thus therapy is of advantage over carbon dioxide and oxygen in that no special apparatus is required, and the immediate attendance of the physician is only necessary for a few minutes.

CONCLUSION

We have determined the effect of the glucose and insulin on the rate of disappearance of alcohol from the blood of intoxicated patients.

- 1 The injection of 15 units of insulin had no effect on the blood alcohol.
- 2 The injection of 50 cc. of 50 per cent glucose intravenously caused a moderately increased fall in blood alcohol only in the severely intoxicated patients. No change was observed in the less severely intoxicated patients.
- 3 The administration of both glucose and insulin accelerated the decrease in blood alcohol in all patients. It is suggested that the oxidation of alcohol may be catalyzed by the simultaneous oxidation of glucose.

BIBLIOGRAPHY

- 1 Solimann T. H., *Manual of Pharmacology* W. B. Saunders Co., Philadelphia, 1926 3rd ed., pp. 654-668.
- 2 Atwater W. O., and Benedict, F. G., An experimental inquiry regarding the nutritive value of alcohol. *Mem. Nat. Acad. Sc.*, 1902, 8 231.
- 3 Haggard, H. W., and Greenberg L. A., Studies in absorption, distribution, and elimination of ethyl alcohol. *J. Pharmacol. & Exper. Therap.*, 1934 52, 137.

tive determination in the photoelectric densitometer (Evelyn) of the amount of unknown necessary to produce a turbidity equal to that produced by a standard extract. The standard deviation in any series is 7.2 per cent. (4) They calculate their results *per volume of blood*, whereas we calculate ours *per volume of corpuscles*. Since blood *V*-factor is confined to the corpuscles, as shown for the *DPN* moiety by Euler and Nilsson (15) and for the entire *V*-factor by Kohn (8), comparison of the various assays of Vilter, Vilter, and Spies (10) must be inaccurate to at least the extent by which the hematocrits differ—which in ordinary hospital work may easily be 100 per cent.

The results obtained are expressed in *de* or *DPN equivalents*. An assay of 10 *de* signifies that 1 ml of corpuscles has a growth promoting activity equal to that of 10 gamma of *DPN* (cozymase). It is to be emphasized, however, that this is an arbitrary measure, since the relative contribution made by each coenzyme (and possibly by unknown related substances) has not been determined. The sensitivity of the method is such that under the usual conditions visible growth is obtained at a dilution of cozymase about 1 part in 500 million, or more. The quantitative estimations can be carried out at about half this dilution with a standard deviation of about 7 per cent in any series. We are greatly indebted to Prof J R nnstrom and Drs A. L nerstrand and E. Sperber of Stockholm for a generous gift of *DPN* (63 per cent purity) with which we have calibrated our standards.

Since the specificity, theory, and procedure of the test have already been discussed in detail (8), it is only necessary to give here a resume of our technic, indicating the improvements which have been introduced. It should also be noted that the pH of the blood extract is such that the reduced form of the coenzymes is inactivated. The amount of the reduced form present, however, is usually less than 10 per cent, when estimated as the increase in assay following the addition of $K_3Fe(CN)_6$ to the laked cells previous to trichloroacetic acid precipitation.

Blood is obtained by puncturing the finger tip with a surgical blade (Crescent 111) and gently pressing out 4 or 5 drops into the depression of a small paraffin block, 0.2 ml are then added to 0.8 ml. of isotonic saline (17 volumes of 0.9 per cent NaCl + 1 volume of 2.2 per cent $K_2C_2O_4 \cdot H_2O$). Such diluted blood can be kept at room temperature for 2 hours or at 5° for 5 hours without loss of coenzyme.

For the hematocrit, 0.4 ml of diluted blood are centrifuged in a special pipette, 13.5 cm. long, illustrated in Figure 1a. The walls are of pyrex, about 3 mm. thick, and the bore of the graduated tube is of the order of 1 mm. After filling, the ends are wiped dry, each is sealed with a small piece of adhesive tape, and the whole pipette is enclosed within a rubber band (Goodrich Number 84), the ends being taped as in Figure 1b. The rubber band acts as a shock absorber and permits a half dozen pipettes

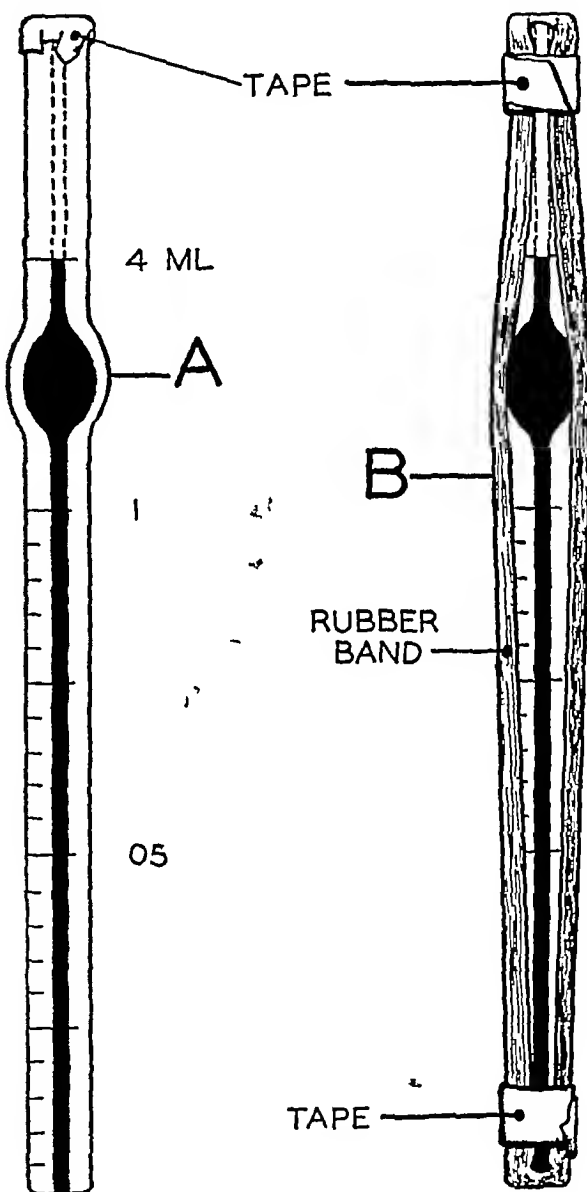


FIG 1 THE HEMATOCRIT PIPETTE

After filling, the ends are wiped dry and each is sealed with a small piece of adhesive tape as shown at the top of A. The pipette is then encased in a rubber band which is held in place by two rings of tape, as in B.

to be spun in one large centrifuge cup. After centrifuging at 2000 r.p.m. for 15 minutes, the volume of the packed cells is read directly from the calibrated stem. These cells can be used for analysis, if desired, by removing the tape and blowing out the contents of the pipette. Ordinarily however an aliquot of diluted blood is used for the blood extract.

Blood extract is prepared by laking 0.2 ml. (or more, if necessary) of diluted blood in 7.8 ml. of distilled water and adding within several minutes 2 ml. of partially neutralized trichloroacetic acid. The test tube is then corked, inverted (to sterilize) centrifuged, and stored in the cold. The clear (or almost clear) supernatant liquid is tested. The strength of the trichloroacetic acid used is about 13.5 per cent, and its pH is 2.1 to 2.2 due to the addition of NaOH. After its addition to the laked blood, the pH of the extract is about 3, which gives optimal protein precipitation.

The broth employed for both stock cultures and tests contains 2 per cent proteose peptone, 0.6 per cent NaCl, 0.1 per cent sucrose, and 0.04 per cent fumaric acid, titrated with NaOH to pH 7.8 (glass electrode). To prevent unnecessary darkening it is autoclaved for not more than 15 minutes at 15 pounds pressure. It is superior to the broth used formerly in that it permits a comparison of the unknown against the standard at an α_2 (turbidity) of 15 or less with a single reading 20 to 24 hours after inoculation. For clinical determinations, only the standards need be made up in duplicate. The stock cultures are transferred daily. For every assay to a series of tubes each containing 7 ml. of broth, there are added 0.0, 0.1, 0.2, and 0.3 ml. of blood extract. The tubes are then inoculated, and the turbidity is finally determined and compared with that produced by a standard *V* factor solution as previously described in detail (8).

RESULTS

The blood coenzyme (*de*) values for a group of 126 hospital patients, selected for the most part

at random, were determined and compared with those for a group of 53 normals. The results are summarized in Figure 2 in the form of frequency distribution polygons which show the percentage of cases having a *de* of 50 to 60, of 60 to 70, of 70 to 80, etc. It is apparent that the *de* values of the clinical subjects have a greater spread than the normal, although the mode of each group falls in about the same range. Of the controls, 64 per cent fall between 50 and 70 *de* and 49 per cent between 60 and 80 *de* of the clinical subjects, 47 per cent fall into each of these classes. The peak between 60 and 70 *de* in the clinical distribution, which is not seen in the controls, may be due to the greater number of females in the clinical series (46 per cent compared to 15 per cent). The female mode falls between 60 and 80 *de*, whereas that for the males lies between 50 and 70 *de*.

The greater spread of the *de* values in the hospital population indicates that the normal distribution has been distorted by an increase in the relative number of high and low values. We have found that diabetics tend to be low and patients suffering from pulmonary disease (pneumonia) tend to be high whereas cases of cardiovascular disease tend to have a normal distribution. Probably most other conditions have essentially normal distributions since, on the whole the polygon for the clinical subjects is similar to that for the controls. Similar results were obtained in an earlier series of 65 cases, which are not reported however, since the technique employed was somewhat different.

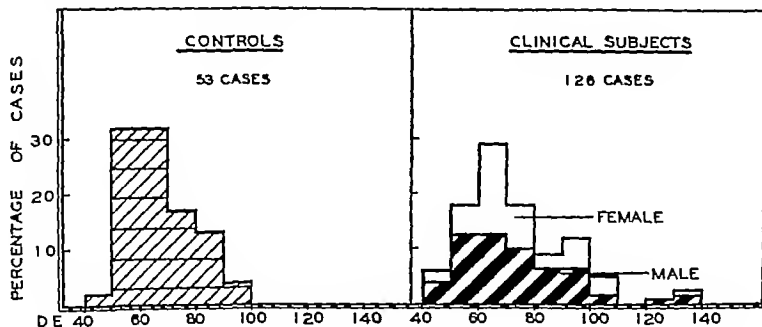


FIG. 2. THE DISTRIBUTION OF *V* FACTOR (*de*) LEVELS IN NORMAL AND PATHOLOGICAL SUBJECTS

Diabetes mellitus

The distribution of 23 cases is shown in Figure 3, where 2 peaks are seen. Thirty per cent of the cases have significantly low values of 40 to 50 *de*. About 50 per cent fall in the definitely normal range of 50 to 80, while the remainder tend to be high. Vilter, Vilter, and Spies (11) examined 3 diabetics in acidosis and found their initial values to be low, when 2 of them were treated with large quantities of insulin and were given other routine therapy, an increase in assay of well over 100 per cent was noted within 10 days. Red cell counts were around 5,000,000. It was therefore possible that the peaks in our series could be correlated with the amount of treatment received. This, however, was not the case. The average *de* for 11 patients who had received no insulin was 55, which was within a few per cent of the average of the insulin-treated cases. Further examination of the individual data revealed no connection between insulin therapy or blood sugar concentration and *de* level. In 2 cases there was actually a drop of 20 to 25 per cent in blood *de* after 10 days of insulin administration. Our results show, therefore, that the diabetic population has a larger percentage of low *de* values than the normal, which is consistent with the findings of Vilter, Vilter, and Spies. The differences noted by us, however, are smaller, and the administration of insulin seems to be unaccompanied by any dramatic effect. This may be accounted for by the fact that we have not examined diabetics during and after recovery from extreme acidosis. Also, the growth factor specificity of the *para-influenza* bacillus may differ from that of the *influenza bacillus* used by Vilter *et al*.

Pulmonary disease

The distribution of 15 cases is shown in Figure 3. The tendency to high *de* values in this group is due to the cases of pneumonitis (cross hatched) rather than to those of tuberculosis (solid). All of the pneumonitis cases except one had been treated with sulfapyridine for at least several days before the blood tests were made. The temperatures had dropped to around 38° and the patients were on the way to recovery. The untreated case was one of bronchopneumonia, had

a temperature of 40°, and a *de* of 79. There are also included with this group 1 case of pleural effusion and 1 of lung abscess. All of the tuberculosis cases were active.

Pellagra

Four pellagrins tested before treatment were reported (8) to be within 20 per cent of 6 normals, the assays being expressed in arbitrary units. The assays converted into *de*, on the basis of re-testing three of the normals, are 50, 50, 54, and 68. Recalculated *de* values for 3 other pellagrins, not previously reported, are adult female, moderately ill, 61, adult male, acutely ill, 52, 10-year-old boy, acutely ill, 70. The tests were made before nicotinic acid was given and while the patients were still sensitive to sunlight. Two more cases are included in the present series, both adult males, a very mild one, *de* 64, and a severe one, *de* 55. All of these results are consistent with the conclusion that pellagrins are only moderately low, falling in the lower range of the normal distribution. The very low values found by Vilter *et al* (10) may be explained, at least in part, by the error in their method of comparison (*cf* Methods). It is also possible that other differences in technic, particularly the use of another species of bacterium, may lead to divergent results.

Cardiovascular disease and others of normal distribution

The distribution of 15 cases of cardiovascular disease is shown in Figure 3. Other groups which appeared normal in distribution were 8 cases of gastro-intestinal disorders (including ulcers and cholecystitis), 8 of psychoses of various types, 5 of syphilis, and 4 of thyroid dysfunction (3 hyper, 1 hypo). Vilter *et al* (11) reported an extremely low value for a case of chronic lymphatic leukemia. The 3 cases examined by us were not definitely abnormal.

Miscellaneous cases

It is impossible to draw conclusions concerning the other diseases where only 1 or 2 cases were examined. However, 2 cases were found with definitely abnormal *de* values in the vicinity of 30. They have not been included in the above sta-

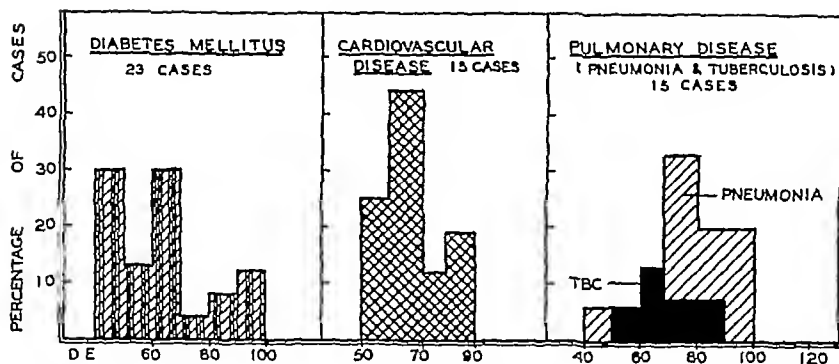


FIG 3 THE DISTRIBUTION OF *V* FACTOR (*de*) LEVELS IN DIABETES MELLITUS, CARDIOVASCULAR DISEASE, AND PULMONARY DISEASE

The cardiovascular disease shows a normal distribution, the diabetes shows a significant percentage of low values and the pulmonary disease a significant percentage of high values.

tistics Both were women, the first having a generalized type of osteomalacia which was refractory to all treatment, and the second having sprue. The low value in the sprue case is not characteristic, since 2 others showed *de* values of 84 and 57 respectively. Both responded as readily and in the same way as normals and pellagrins to the administration of nicotinic acid, the *de* value increasing during administration, but falling back when dosage was discontinued. Although the value could be raised far beyond the normal no clinical improvement resulted. Like the diabetics, these cases show that low *de* values are possible in conditions which are not specifically improved by nicotinic acid and emphasize the discreteness and independence of the factors which control the *de* level. In this connection, the case of a normal individual is worth mentioning. We have found the *de* level to be quite constant when followed for fairly long periods of time (several months). A striking exception to this is shown in Figure 4 where 2 healthy, adult males are compared over a period of 65 days. The variation of Subject A who served as a control was about ± 3 per cent, whereas Subject B varied between 84 and 63 *de*. Nevertheless Subject B enjoyed excellent health and his diet and activities experienced no unusual variation.

Among the hospital patients in Figure 2 there is a small group which falls between 120 to 140 *de*. This includes 1 case of pernicious anemia (131 *de*) which had received 30 USP units of liver (Lederle) intramuscularly daily for 7 days. During this period the erythrocyte count rose from 14 to 1.85 million and the reticulocyte count rose from 0.2 to 29 per cent. On the basis of other studies (9), it is most probable that the high reticulocyte count is responsible for the high assay in this case. The other 2 cases cannot be

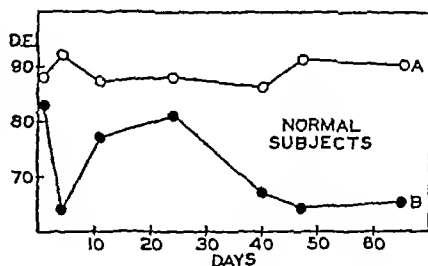


FIG 4 THE *V* FACTOR (*de*) LEVELS IN TWO NORMAL INDIVIDUALS

A is typical in showing a relatively constant level. B is atypical, showing a marked variation which is not correlated with any change in condition.

explained. One was diagnosed as epilepsy probably of organic origin (125 *de*), the other was one in which the final clinical impression was menopausal syndrome and malnutrition (134 *de*). For comparison, it may be noted that a case of idiopathic epilepsy had an assay of 62 *de*.

DISCUSSION

The present work emphasizes the great variability in *de* existing in a population of normal individuals, differences of the order of 50 per cent being found frequently. The small variation previously reported (8) in a group of 6 individuals was probably due to homogeneity with respect to age, sex, and occupation. In spite of this variation, however, we now know of one factor which directly affects the *V*-factor level, namely, nicotinic acid, of two associated with depression, diabetes mellitus, and pellagra, and of one associated with elevation, pulmonary infection.

In addition, however, it seems necessary to postulate at least one other factor which operates independently of the above in order to account for (a) the extremely high and low assays, (b) the normal variation, and (c) the type of instability shown by Subject B, Figure 4. The relative stability of the blood level (which is extreme in Subject A, Figure 4) was noted previously (8) and formed the basis for the suggestion that it is determined by the general condition and relationships of the hematopoietic mechanism. On the other hand, the increment following nicotinic acid dosage (and which disappears when dosage is discontinued) can be accounted for as a corpuscular phenomenon and can be duplicated *in vitro* (9). Such an hypothesis implies that the blood level depends not only upon the current state of nutrition, but also upon the condition of the myeloid apparatus, and upon its power of abstracting needed substances from other loci. To judge by our results, the nutritional factor is of some but not of great importance in man, and the same is true of the dog. It also seems that considerable latitude is possible in the relations involved without danger to the organism. These considerations lead to the obvious conclusion that the *de* level, as determined by our technic, can be of little importance for diagnosis or prognosis.

SUMMARY

The *V*-factor (coenzymes one and two, possibly plus unknown related substances) level was determined for the blood of 53 normal individuals and 126 hospital patients. The assays are expressed in *de* or activity equivalent to gamma of coenzyme one per ml of corpuscles. Eighty-one per cent of the normal cases fell between 50 and 80 *de* whereas only 65 per cent of the pathological cases fell in this range. The greater variation in the pathological series was caused, to a large extent, by the diabetics who showed low values and the patients with pulmonary disease who showed high values. Pellagrins showed values that were in the lower part of the normal range. To account for all of the variation observed, it is necessary to postulate factors other than diet or disease which regulate the blood *de*. An earlier series of 65 cases, not reported in detail, is consistent with these conclusions.

BIBLIOGRAPHY

- 1 Euler, H. v., Die Cozymase. *Ergebn. d. Physiol.*, 1936, 38, 1.
- 2 Warburg, O., Chemische Konstitution von Fermenten. *Ergebn. d. Enzymforsch.*, 1938, 7, 210.
- 3 Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolley, D. W., The isolation and identification of the anti-black tongue factor. *J. Biol. Chem.*, 1938, 123, 137.
- 4 Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H., Treatment of human pellagra with nicotinic acid. *Proc. Soc. Exper. Biol. and Med.*, 1937, 37, 405.
- 5 Smith, D. T., Ruffin, J. M., and Smith, S. G., Pellagra successfully treated with nicotinic acid, a case report. *J. A. M. A.*, 1937, 109, 2054.
- 6 Harris, L. J., Vitamin B₃ complex, further notes on "monkey pellagra" and its cure with nicotinic acid. *Biochem. J.*, 1938, 32, 1479.
- 7 Chick, H., Macrae, T. F., Martin, A. J. P., and Martin, C. J., Experiments with pigs on a pellagra producing diet. *Biochem. J.*, 1938, 32, 844.
- 8 Kohn, H. I., The concentration of coenzyme-like substance in blood following the administration of nicotinic acid to normal individuals and pellagrins. *Biochem. J.*, 1938, 32, 2075.
- 9 Kohn, H. I., and Klein, J. R., The synthesis of cozymase and of *V*-factor from nicotinic acid by the human erythrocyte *in vitro* and *in vivo*. (In press.)
- 10 Vilter, R. W., Vilter, S. P., and Spies, T. D., Relationship between nicotinic acid and a codehydro-

- genase (cozymase) in blood of pellagrins and normal persons. *J. A. M. A.*, 1939 112 420
- 11 Vilter R. W., Vilter S. P., and Spits, T. D. Determination of codehydrogenases I and II (cozymase) in the blood of diabetics in severe acidosis. *Am. J. M. Sc.*, 1939 197, 322.
- 12 Axelrod, A. E., and Elvehjem, C. A., Effect of nicotinic acid deficiency on the cozymase content of tissues. *Nature*, 1939, 143 281
- 13 Kohn, H. L., and Dann, W. J., Blood *V* factor in the normal and black tongue dog. *Am. J. Physiol.* (Proceedings) 1939 (In press.)
- 14 Lwoff A. and Lwoff M., Studies in codehydrogenases nature of growth factor "*V*" *Proc. Roy. Soc., London, B.*, 1937 122 352, 360
- 15 Euler H. v., and Nilsson, R., Co-zymase Bestimmungen der Co-zymase im Blut. *Ztschr. f. physiol. Chem.*, 1926 162, 63

TREATMENT OF GAS GANGRENE INFECTIONS IN GUINEA-PIGS WITH NEOPRONTOSIL, SULFANILAMIDE, AND SULFAPY- RIDINE AN EXPERIMENTAL STUDY

By DOUGLAS B KENDRICK, JR.¹

(From the Institute of Experimental Medicine The Mayo Foundation Rochester Minn.)

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The development at the end of the World War of an antitoxin for the prophylaxis and treatment of gas gangrene infections provided a method which, in the hands of some men, has been of great value in helping to reduce the mortality rate of this infection. Ghormley (1) has called attention to the beneficial results obtained by the use of antitoxin. He reported a mortality rate of 42.5 per cent in gas gangrene infections which compares favorably with the report of the Surgeon-General's Office of a rate of 48.52 per cent in the American Expeditionary Force in France. However, Ghormley stated that 86 per cent of 33 patients with gas gangrene infections recovered following the use of antitoxin. He stressed the point that antitoxin is of value in the treatment of this infection when given early. Stone and Holsinger (2) reported 67 cases of gas infection with a mortality rate of 32.4 per cent. When antitoxin was used the rate was reduced to 15.3 per cent. On the other hand Boland (3), reporting a group of 15 cases of gas infection associated with compound fractures found that when antitoxin was used 4 of these patients died and 4 lived when antitoxin was not used 2 died and 5 lived. However, the number of cases is too small to be statistically significant. Bohleman (4) reported disappointing results when gas gangrene infections were treated with antitoxin.

Owing to the discrepancies found in the literature on the results obtained when antitoxin was used in gas gangrene infections one is led to believe that it is far from being dependable in the majority of cases of the infection. For this reason many attempts have been made to find other methods of treatment. Sulfanilamide has been tried by several workers for this purpose. Bohleman (4) reported 3 cases of gas gangrene infection treated with sulfanilamide without a fatal

ity. These patients had received 10 000 units of combined gas bacillus antitoxin on admission but were given no antitoxin after the diagnosis of gas gangrene was made. The presence of *Clostridium welchii* in the wounds of 2 of the patients was not confirmed by laboratory methods. Treatment consisted primarily of sulfanilamide by mouth for several days. Mellon Gross, and Cooper (5) reported one case of gas gangrene treated with sulfanilamide. This patient had a chronic prostatic disease and a severe diabetes, and a gas infection had developed as the result of catheterization. Large doses of sulfanilamide failed to improve the infection. Long and Bliss (6) working on gas gangrene infection in mice produced experimentally by injecting washed cultures of *Clostridium welchii* have shown that sulfanilamide protects mice infected with *Clostridium welchii* very effectively. In 3 experiments in which *Clostridium welchii* was resuspended in broth, 36 of 50 treated mice (72 per cent) survived. Only 2 out of 27 untreated mice, used as controls survived the injection of 0.5 cc. of the culture. The results obtained by these workers, using unwashed cultures were disappointing.

The results of a series of experiments to determine the protection afforded by sulfanilamide, neoprontosil, and sulfapyridine in the treatment of gas gangrene infections produced experimentally in guinea pigs are here reported.

METHODS

The cultures of *Clostridium welchii* used in this work were obtained from 2 different sources. The first strain was collected from a nonfatal case of gas gangrene in a human. The second strain was obtained from an elk that had died from a gas gangrene infection. The organisms were isolated from the spleen of the elk and transferred to brain broth mediums for culturing. Cultures incubated at 37° C. for 24 hours were used in all experiments. Intramuscular injections were done routinely.

In order to determine the smallest quantity required to

¹ Captain Medical Corps United States Army on assignment in The Mayo Foundation.

produce a fatal infection consistently, it was necessary to inject graduated amounts of the culture into several guinea-pigs. Three guinea-pigs were injected with 10, 0.5 and 0.1 cc. of the culture respectively and 3 guinea-pigs were injected with 0.1 cc. of the culture mixed with 0.1 cc. of 1-1000 adrenalin. The adrenalin was used to produce a local anoxia so as to increase the chance of an infection developing. The guinea-pigs were dead in 24 hours from extensive gas gangrene infections. Further, to test the amount required to produce fatal infections, cultures were made directly from the guinea-pigs that had died in the previous experiment, and after incubating for 24 hours 0.1 cc. of each culture mixed with 0.1 cc. of 1-1000 adrenalin was injected into 4 guinea-pigs. Three of the guinea-pigs died from the infection and the fourth, which had been injected with a culture made from the guinea-pig receiving 0.1 cc. of culture primarily, had a sloughing lesion of the thigh, but it was not fatal. From these experiments we found that 0.1 cc. of the culture mixed with 0.1 cc. of 1-1000 adrenalin would produce fatal lesions regularly.

There is a great deal of variation in the dosage of sulfanilamide advocated for use in small animals. Mellon, Gross, and Cooper (7) recommended giving mice orally doses as small as 0.3 gram and as large as 1.25 grams per kilogram for a period of several days. They considered the large dose to be preferable. Kolmer, Raiziss, and Rule (8) suggested giving sulfanilamide to rabbits in doses varying from 0.1 to 0.5 gram per kilogram orally twice daily. The tolerance of guinea-pigs for sulfanilamide was tested by administering the drug in graduated doses, so that each pig received a different amount. The doses varied from 0.5 gram to 2.0 grams per kilogram. Among the guinea-pigs receiving 2.0 grams per kilogram, marked cyanosis and spastic paralysis of the spinal muscles developed, resulting in death within 24 hours. Doses of 1.5 and 1.0 grams per kilogram caused cyanosis but no paralysis. The pigs receiving 0.5 gram per kilogram showed no untoward results. When 250 mgm. of sulfanilamide in 20 cc. of physiological saline solution was injected subcutaneously and intraperitoneally into guinea-pigs, all animals survived, it was, therefore, thought safe to use this dose. In some experiments 350 mgm. doses were used perorally twice daily.

Neoprontosil was used initially to determine its therapeutic value in gas gangrene infections. It was injected subcutaneously in 250 mgm. doses twice at 6-hour intervals. In this series, as in all the experiments, the first dose of the drug was given when the culture of *Clostridium welchii* was injected. Sulfanilamide was next employed to determine its efficacy. It was given orally, subcutaneously, and intraperitoneally in doses varying from 250 to 350 mgm. twice daily. Finally, sulfapyridine was used to combat the gas infection. Two hundred and fifty milligrams of the drug were given by mouth twice daily.

In the preceding experiments unwashed cultures had been used, owing to the overwhelming infections produced it was thought advisable to use washed cultures to

reduce the quantity of preformed toxins in the cultures as suggested by Long and Bliss (6) and by Long (9). A 24-hour brain broth culture (from elk) was washed several times in physiological saline solution by centrifugation at 1500 r.p.m. for 30 minutes. Then the organisms were diluted to the original volume with physiological saline solution. Then 0.1 cc. of the washed culture, mixed with 0.1 cc. of 1-1000 adrenalin, was used as the inoculating medium.

RESULTS

Ten guinea-pigs were inoculated intramuscularly with a 24-hour brain broth culture of *Clostridium welchii* mixed with 0.1 cc. of 1-1000 adrenalin. Five of the guinea-pigs were treated with 500 mgm. of neoprontosil subcutaneously in 2 doses given 6 hours apart. Five guinea-pigs were used as controls. The guinea-pigs in the treated group were dead within 48 hours. Those in the control group were dead within 6 days.

Ten guinea-pigs were inoculated with the standard dose of a culture of *Clostridium welchii*, 3 were given the culture mixed with 0.1 cc. of 1-1000 adrenalin and 3 received the culture without adrenalin. Of the 4 controls, 2 received culture mixed with adrenalin and 2 received the culture without adrenalin. The treated group were given 250 mgm. of sulfanilamide at the time the culture was inoculated. Four of the treated group and all of the control group died.

The strain of *Clostridium welchii* used in the remaining portion of the work was obtained from a fatal case of gas gangrene in the elk.

Ten guinea-pigs were injected with 0.1 cc. of culture of *Clostridium welchii*. Sulfanilamide in 300 mgm. doses was given intraperitoneally twice at 6-hour intervals to the treated group. The infection was fatal to the control group in 24 hours and all the treated guinea-pigs were dead in 48 hours.

A comparison of the relative value of sulfanilamide and combined gas gangrene antitoxin was made. Ten guinea-pigs were inoculated with the standard quantity of culture of *Clostridium welchii* mixed with adrenalin. Four of the treated group received 300 mgm. of sulfanilamide subcutaneously twice daily and 3 were given 2000 units of antiperfringens serum when the culture was injected. Three of the guinea-pigs injected

with the drug died by the fifth day, the fourth survived. All of the guinea pigs inoculated with the antitoxin survived. Only one guinea pig in the control group lived.

Ten guinea pigs were inoculated with 0.1 cc. of a 24-hour culture of *Clostridium welchii*. The treated group was given 350 mgm. of sulfanilamide by mouth twice daily. The guinea pigs in the treated group were dead within 48 hours and those in the control group died within 60 hours after inoculation.

Two experiments were done to test the efficacy of sulfapyridine in gas gangrene infections. Ten guinea pigs in each experiment were inoculated with 0.1 cc. of a culture of *Clostridium*

welchii. The treated groups were given 250 mgm. of sulfapyridine by mouth twice daily. In the first experiment only 1 out of 5 guinea pigs in the treated and 1 out of 5 in the control group lived. In the second experiment the infection was fatal to both groups.

Six guinea pigs were inoculated with 0.1 cc. of a washed culture of *Clostridium welchii* mixed with 0.1 cc. of 1-1000 adrenalin. The treated group was given 350 mgm. of sulfanilamide by mouth twice daily. The results were practically the same as when unwashed cultures were used. The guinea pigs in the treated and control groups were dead within 4 days.

A résumé of the results is given in Table I.

TABLE I

Results of administering neoprontosil, sulfanilamide, sulfapyridine, or antiperfringens serum to guinea pigs infected with *Clostridium welchii*

Experiment number	Number of guinea pigs	<i>Clostridium welchii</i> inoculated	Treatment	Number of deaths								Survivals
				1 day	2 days	3 days	4 days	5 days	6 days	7 days	10 days	
Controls	35	0.1 cc.	None	15	5	2	3	4	1	1	2	2
1	5	0.1 cc. of Culture 1†	250 mgm. of neoprontosil subcutaneously twice at 6-hour intervals	2	3							0
2	6	3 guinea pigs given 0.1 cc. of Culture, 1 with adrenalin. 3 guinea pigs given 0.1 cc. of culture, 1 with out adrenalin	250 mgm. of sulfanilamide intraperitoneally Only 1 dose injected								4	2 (They received culture only)
3	5	0.1 cc. of Culture 2	300 mgm. of sulfanilamide intraperitoneally twice at 6-hour intervals	4	1							0
4	4	0.1 cc. of Culture 2	300 mgm. of sulfanilamide subcutaneously twice daily	1	1			1				1
5	5	0.1 cc. of Culture 2	350 mgm. of sulfanilamide orally twice daily	1	4							0
6	10	0.1 cc. of Culture 2	250 mgm. of sulfapyridine orally twice daily	6	3							1
7	3	0.1 cc. of Culture 2 washed	350 mgm. of sulfanilamide orally twice daily		1		2					0
Total *	38			14	13	0	2	1	0	0	4	4
4	3	0.1 cc. of Culture 2	2000 units antiperfringens serum injected subcutaneously once									3

* Treated with neoprontosil, sulfanilamide, or sulfapyridine.

† Culture 1 obtained from a nonfatal case in a human. Culture 2 obtained from a fatal case of gas gangrene in elk.

SUMMARY

A series of experiments has been done to study the therapeutic value of neoprontosil, sulfanilamide, and sulfapyridine in gas gangrene infections produced experimentally in guinea-pigs. The results obtained with the 3 drugs were very similar. In the doses used, whether the cultures were washed or unwashed, these drugs did not provide protection against the infection. In the treated group there was a mortality rate of 89.5 per cent, while in the control group the rate was 94.3 per cent.

A comparison has been made between the protective value of antiperfringens serum and sulfanilamide in gas gangrene infections in guinea-pigs. The antiperfringens serum proved to be much more effective. In spite of the small number of guinea-pigs treated with the serum the difference in results is statistically significant.

REFERENCES

- 1 Ghormley, R. K., Gas gangrene and gas infections. *J Bone and Joint Surg*, 1935, 17, 907
- 2 Stone, C. S., Jr, and Holsinger, H. B., Diagnosis and treatment of gas bacillus infection. *Virginia M. Monthly* 1934, 61, 200
- 3 Boland, F. K., Gas gangrene in compound fractures. *Ann. Surg.*, 1929, 90, 603
- 4 Bohlman, H. R., Gas gangrene treated with sulfanilamide, report of three cases. *J. A. M. A.*, 1937, 109, 254
- 5 Mellon, R. R., Gross, Paul, and Cooper, F. B., Sulfanilamide therapy of bacterial infections with special reference to diseases caused by hemolytic streptococci, pneumococci, meningococci and gonococci. Charles C. Thomas, Springfield, Illinois, 1938, pp 5, 217
- 6 Long, P. H., and Bliss, E. A., Observations upon experimental and clinical use of sulphaniilamide in treatment of certain infections. *Canad. M. A. J.*, 1937, 37, 457
- 7 Mellon, R. R., Gross, Paul, and Cooper, F. B., Sulfanilamide therapy of bacterial infections with special reference to diseases caused by hemolytic streptococci, pneumococci, meningococci and gonococci. Charles C. Thomas, Springfield, Illinois, 1938, p 33
- 8 Kolmer, J. A., Raiziss, G. W., and Rule, Anna M., Sulfanilamide and diaminodiphenylsulfone and their diacetyl derivatives in treatment of experimental intradermal streptococcus infections of rabbits. *Proc. Soc. Exper. Biol. and Med.*, 1938, 39, 95
- 9 Long, P. H. Personal communication through Colonel Siler to the author

ULTRAFILTRABLE MAGNESIUM IN HYPERTHYROIDISM

By LOUIS J. SOFFER, D. ALFRED DANTES¹, EDWARD B. GROSSMAN,
HARRY SOBOTKA AND MILDRED D. JACOBS

(From the Medical Service of Dr. George Baehr and the Division of Laboratories of The Mount Sinai Hospital, New York City)

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The following report is concerned with the study of magnesium metabolism in clinical and experimental hyperthyroidism. This first communication deals primarily with the ratio of diffusible magnesium to total serum magnesium in the hyperthyroid state.

The general question of relationship of total serum magnesium to its ultrafiltrable fraction in 8 normal individuals has been discussed by Watchorn and McCance (1). They found that normally approximately 25 per cent of the total serum magnesium is non-diffusible. These authors suggest that there are probably 2 factors which play a part in determining the amount of ionizable magnesium. The bound magnesium may be in combination either with the protein in the serum or with the serum phosphatides. These acids which behave similarly to protein, are present in adequate quantities to bind considerable amounts of magnesium. Brull (2) and Scholtz (3) also believe that both calcium and magnesium form colloidal phosphate complexes.

METHOD

The total serum magnesium was determined by the method of Briggs (4). The serum proteins were precipitated with trichloroacetic acid. It was found that little, if any magnesium was carried down with the protein flocculum. To 10 cc. of protein free filtrate were added 1 cc. of 20 per cent sodium acetate, 6 to 8 drops of 0.016 per cent bromocresol green and 1 cc. of 4 per cent ammonium oxalate. The pH of the solution was adjusted to 5.0 by addition of ammonium hydroxide. The mixture was allowed to stand overnight, and the precipitated calcium oxalate then separated by centrifugation. To the decanted supernatant fluid and washings were added 1 cc. of 2 per cent potassium dihydrogen phosphate,

and 1 cc. of concentrated ammonia solution. After the mixture had again been allowed to stand overnight, the precipitate was separated by centrifugation and washed with a solution containing 200 cc. of 95 per cent alcohol and 50 cc. of concentrated ammonia solution per liter. The precipitated magnesium ammonium phosphate was dried and determined according to the method of Kuttner and Lichtenstein (5) by comparison of the color developed on addition of 7.5 per cent sodium molybdate and 0.2 per cent stannous chloride with that of a standard phosphate stock solution.

For the determination of diffusible magnesium serum was ultrafiltered through a "600" Cellophane membrane under a pressure of 80 pounds nitrogen per square inch. The magnesium content of the ultrafiltrate was determined as described above, except that the protein precipitate with trichloroacetic acid was omitted.

RESULTS

Total and ultrafiltrable magnesium determinations were made in normal individuals in patients with neurocirculatory asthenia, various muscular dystrophies, and in patients with clinically well defined hyperthyroidism. In many of the latter group determinations were made before and after the administration of Lugol's solution and after operation.

In Table I are presented the results obtained in 14 normal individuals. It was found that the percentage of the total serum magnesium which is non-diffusible varied in these individuals from 31 to 221 per cent, the largest number of the group falling between 10 and 19 per cent. The total serum magnesium varied between 2.12 and 2.76 mgm per cent with one exception of 4.30 mgm per cent.

In Table II are included the data obtained in patients with neurocirculatory asthenia. The

¹ Aided by a grant from the Herbert Celler Fund.

TABLE I

Total serum magnesium and percentage of bound magnesium in normal individuals

Number	Total protein	Total magnesium	Magnesium in ultrafiltrate	Percentage of bound magnesium
	grams per cent	mgm per 100 cc	mgm per 100 cc	per cent
1	7.0	2.12	1.83	13.7
2	8.4	2.55	2.47	3.1
3	8.2	2.36	2.06	12.7
4	7.8	2.27	2.19	3.5
5	7.5	2.15	1.72	20.0
6	7.0	2.76	2.20	20.3
7		4.30	3.75	12.8
8		2.23	2.01	9.9
9		2.46	2.00	18.7
10		2.33	1.93	17.2
11		2.33	2.17	6.9
12		2.58	2.04	20.9
13		2.60	2.04	21.5
14		2.21	1.72	22.1
Average	7.6	2.52	2.15	14.5

TABLE II

Total serum magnesium and percentage of bound magnesium in neurocirculatory asthenia and various myopathies

Number	Total magnesium	Magnesium in ultrafiltrate	Percentage of bound magnesium
	mgm per 100 cc	mgm per 100 cc	per cent
NEUROCIRCULATORY ASTHENIA			
1	2.60	2.10	19.2
2	2.33	1.91	18.0
3	2.47	2.02	18.2
4	2.96	2.35	20.6
5	3.48	3.16	9.2
PROGRESSIVE MUSCULAR DYSTROPHY (HYPERTROPHIC)			
1	2.60	1.85	28.8
2	2.35	2.16	8.1
MYASTHENIA GRAVIS			
1	2.34	2.09	10.7
2	2.51	1.96	21.9
3	2.70	2.28	15.5
4	2.67	2.04	23.6
5	2.33	2.17	6.9

percentage of bound magnesium varied between 9.1 and 20.6 per cent, while the total serum magnesium varied between 2.33 and 3.48 mgm per cent.

The results obtained in this group are almost identical with those found in the normal individ-

uals. The basal metabolic readings of the patients with neurocirculatory asthenia were all well within the normal limits. It is frequently difficult to distinguish clinically between this group and patients with definite hyperthyroidism. But, as we shall see in the subsequent tables, the behavior of the serum magnesium in normal individuals and in patients with neurocirculatory asthenia is entirely different from that in hyperthyroidism.

In Table II are also presented the results obtained in 7 patients with various myopathies. Five of these patients have myasthenia gravis, while 2 have progressive muscular dystrophy. Again, it will be seen that the results in these patients are identical with those of the normal group, with the exception of one patient with progressive muscular dystrophy who showed some elevation of the percentage of bound magnesium.

The results obtained in patients with Graves' disease were striking. For this study 31 instances of hyperthyroidism of varying intensity were investigated. In each instance the clinical diagnosis was confirmed by microscopic study of the thyroid gland removed at operation. In 13 of these 31 patients total and ultrafiltrate serum magnesium determinations were made before and after the administration of Lugol's solution, in 12 control and postoperative studies were conducted, and finally in 6 instances determinations were made before the administration of iodine, after the administration of Lugol's solution, and after operation. The postoperative specimens were collected 10 days after the operation just prior to discharge from the hospital.

In Table III are presented the data obtained in the 31 patients with hyperthyroidism. The basal metabolic rate in these patients varied between plus 30 and plus 106 per cent. The total serum magnesium in these patients, directly on admission to the hospital, varied between 1.85 and 2.96 mgm per cent, while the percentage bound varied between 21.5 and 61.6 per cent. Overlapping of the percentages of bound magnesium in the normal controls with those in the patients with hyperthyroidism occurred in only 2 instances. The bound magnesium in most of the normals was less than 20 per cent, while among patients with hyperthyroidism values over 30 per cent prevailed. No significant change of total serum

TABLE III

Total serum magnesium and percentage of bound magnesium in patients with hyperthyroidism

Number	Basal metabolic rate	Total protein	Total magnesium	Magnesium in ultra filtrate	Percentage of bound magnesium
	per cent	grams per cent	mgm per 100 cc.	mgm per 100 cc.	per cent
1	+45	8.0	2.16	1.15	46.7
2	+66	7.2	2.64	1.74	34.1
3	+43	6.6	2.10	0.86	60.0
4	+40	7.5	1.85	0.71	61.6
5	+41	7.3	2.54	1.41	44.4
6	+43	7.5	2.79	2.02	27.6
7	+30	7.1	2.64	1.91	27.6
8	+47	7.6	2.11	1.60	24.1
9	+106	7.4	2.24	1.39	38.0
10	+49	5.8	2.22	1.49	32.8
11	+38	7.1	2.02	1.08	46.5
12	+41	6.4	2.60	1.35	48.0
13	+56	7.2	2.30	1.58	41.2
14	+56	7.2	2.62	1.52	42.0
15	+60	6.5	2.46	1.69	31.3
16	+50		2.68	1.37	49.8
17			2.59	1.72	30.0
18	+37		2.42	1.60	33.8
19	+30		2.35	1.54	34.4
20			2.44	1.61	33.6
21	+50		2.41	1.90	21.5
22	+48		2.30	1.70	26.0
23	+42		2.59	1.65	36.3
24	+40		2.71	1.87	31.0
25	+46		2.56	1.69	34.0
26	+36		2.38	1.72	27.7
27			2.55	1.71	32.9
28	+56	7.0	2.75	1.72	37.4
29	+60		2.41	1.73	28.2
30	+40		2.31	1.80	22.2
31	+34		2.96	2.08	30.0
Average		7.1	2.44	1.58	36.0

magnesium occurred in patients with Graves disease as compared to the normals.

It is evident therefore, that in hyperthyroidism there occurs a marked increase in the amount of circulating magnesium which is bound at the expense of the diffusible fraction. There is no correlation between the percentage of bound magnesium and the basal metabolic rate, perhaps due to the fact that the metabolic rate was determined very shortly after admission to the hospital ward and may not represent the true basal rate.

In 13 patients the total and bound magnesium in the blood was determined before and after the administration of Lugol's solution (Table IV). There is no essential change in the total serum magnesium before and after adequate administration of iodine but a considerable change occurred in the non filtrable fraction. Thus, before the administration of Lugol's solution the bound mag-

TABLE IV

Total serum magnesium and percentage of bound magnesium in hyperthyroidism before treatment after the administration of Lugol's solution and after operation

Case number	Before treatment			After iodization			After operation		
	Magnesium			Magnesium			Magnesium		
	Basal metabolic rate	Total	Percentage bound	Basal metabolic rate	Total	Percentage bound	Total	Percentage bound	Percentage bound
	per cent	mgm. per 100 cc.	per cent	per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.	per cent	per cent
13	+41	2.60	1.35	+39	2.40	1.81	2.44		
14	+46	2.80	1.28	+41	2.35	1.66	30.0		
15	+40	2.46	1.80	+33	2.43	1.68	30.8		
16	+30	2.25	1.54	+34	2.51	1.53	21.2		
17	+36	2.41	1.80	+37	2.51	1.82	16.6		
18	+34	2.38	1.72	+37	2.84	2.17	22.1		
19	+40	2.41	1.73	+39	2.50	1.91	22.6		
20	+55	2.63	1.53	+43	2.69	1.78	22.0	2.45	17.4
21	+58	2.68	1.37	+48	2.67	1.83	20.5	2.83	17.6
22	+48	2.30	1.70	+30	2.74	2.35	14.6	2.00	16.4
23	+41	2.71	1.87	+18	2.65	1.81	31.0	1.91	19.1
24	+34	2.55	1.71	+29	2.73	2.03	21.0	2.53	21.0
25	+40	2.96	2.08	+30	2.84	2.20	6.0	2.67	24.5
26	+40	2.31	1.80						
27									
28									
29									
30									
31									

nesium varied between 21.5 and 49.8 per cent, while afterwards the bound fraction varied between 6.0 and 33.0 per cent. After operation the results were even more striking. In 12 patients (Table IV) studies were conducted when they were first admitted to the hospital before receiving iodine, and again just before discharge from the hospital after subtotal thyroidectomy. The non-diffusible fraction before operation varied between 22.2 and 49.8 per cent while postoperatively it varied between 6.0 and 20.5 per cent a return to normal levels. This is again demonstrated in those 6 patients in whom determinations were made during the control period, after the administration of Lugol's solution and after operation (Table IV). After iodine administration some drop occurred in the percentage of bound magnesium which dropped still further to normal levels after the operation.

During the period of this study we have had 2 patients with myxedema. In both patients the basal metabolic rate was minus 40 and in both instances there was no circulating bound magnesium the direct opposite of the finding in pa-

tients with hyperthyroidism. The total serum magnesium in the patients with myxedema was well within the normal range.

The mechanism which produces an increase in the percentage of bound magnesium in hyperthyroidism is obscure, and one may raise the question as to whether the blood colloids play some part in the binding mechanism. The amount of blood protein in our series, however, was no greater in patients with hyperthyroidism than in the normal individuals, so that we must postulate some change in the nature of the blood proteins to explain the increase in the binding mechanism. To investigate this phase we performed the following experiments: from 50 to 200 mgm of thyroglobulin were injected intravenously in one dose into 5 dogs. The total and ultrafiltrable blood magnesium was determined at intervals of 15 minutes, 1, 5, and 24 hours. In each instance the increase in bound magnesium varied between 30 and 100 per cent over the control level (Table V). This increase occurred

injection of the thyroglobulin and the increase in bound magnesium would indicate that there is no direct union between the ion and the protein, but rather that the latter sets into motion some mechanism which causes binding of magnesium. *In vitro* experiments failed to demonstrate any binding between thyroglobulin and magnesium. Further studies are being conducted to determine the various factors which operate to bind the blood magnesium.

SUMMARY

1 Total serum and ultrafiltrate magnesium determinations were made in 14 normal individuals, 5 patients with neurocirculatory asthenia, 7 patients with various muscular dystrophies, and in 31 patients with hyperthyroidism.

2 In the normal individuals the percentage of bound magnesium varied between 31 and 22.1 per cent.

3 The patients with neurocirculatory asthenia and various types of muscular dystrophy showed no change in the percentage of bound magnesium as compared to the normals.

4 The patients with hyperthyroidism showed a marked increase in the non-diffusible fraction of the total blood magnesium. The percentage of bound magnesium in these patients varied between 21.5 and 61.6 per cent.

5 No relationship was noted between the basal metabolic rate and the percentage of bound magnesium.

6 Following iodolization there is a considerable decrease in the non-diffusible magnesium fraction with a further drop and return to normal levels after thyroidectomy.

7 In two patients with myxedema, there was no circulating bound magnesium.

8 Thyroglobulin injected intravenously into dogs produces a considerable transient increase in the non-diffusible magnesium fraction suggesting that this protein plays some part in the binding mechanism.

9 The determination of the percentage of bound magnesium may serve as a means for diagnosing obscure and borderline instances of hyperthyroidism.

TABLE V

Percentage of bound magnesium following the injection of thyroglobulin, thyroxine and horse serum intravenously into dogs

No.	Dose	Percentage of bound magnesium				
		Control	15 minutes	1 hour	5 hours	24 hours
1	Thyroglobulin 50 mgm	23.0	36.8	31.7	46.5	15.8
2	Thyroglobulin 100 mgm	25.5	23.4	51.0	24.5	17.0
3	Thyroglobulin 100 mgm	27.0	15.3	33.3	35.0	27.5
4	Thyroglobulin 200 mgm	29.1	22.8	32.8	45.6	37.5
5	Thyroglobulin 200 mgm	15.2	26.2	23.5	24.6	6.5
6	Thyroxine 5 mgm	13.7	5.4	9.5	5.0	11.6
7	Horse serum 5 cc	7.3	7.3	8.2	7.5	7.0

within 1 to 5 hours after the injection. The injection of equivalent doses of thyroxine and horse serum produced no change in the percentage of bound magnesium. This would suggest that the thyroglobulin plays some rôle in binding magnesium and would further suggest that there is an increase of this substance in the blood of patients with hyperthyroidism. The fact that a considerable period of time elapses between the

BIBLIOGRAPHY

- 1 Watchorn, Elsie, and McCance, R. A., Inorganic constituents of cerebrospinal fluid ultrafiltration of calcium and magnesium from human sera. *Biochem. J.*, 1932, 26 54
- 2 Brull, Lucien, L'excrétion des phosphates par le rein et sa régulation. *Archives Internat. de Physiologie*, 1928, 30 1
- 3 Scholtz, Hans Georg Über Änderungen des physikalischen Zustandes von anorganischen Bestandteilen des Serums durch gegenseitige Beeinflussung *Biochem. Ztschr.*, 1931 231, 135 *Arch. f. exper. Path. u. Pharmacol.*, 1930 157 133
- 4 Briggs, A. P., Colorimetric method for determination of small amounts of magnesium. *J. Biol. Chem.*, 1922, 52, 349
- 5 Kuttner T., and Lichtenstein, L., Micro colorimetric studies. II Estimation of phosphorus molybdic acid stannous chloride reagent. *J. Biol. Chem.*, 1930 84, 671

THE COAGULATION DEFECT IN HEMOPHILIA THE CLOT PROMOTING ACTIVITY IN HEMOPHILIA OF BERKEFELDED NORMAL HUMAN PLASMA FREE FROM FIBRINOGEN AND PROTHROMBIN¹

By EUGENE L. LOZNER, ROBERT KARK, AND F. H. L. TAYLOR

(From the Thorndike Memorial Laboratory Second and Fourth Medical Services (Harvard) of the Boston City Hospital and the Department of Medicine, Harvard Medical School Boston)

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Previous investigations in this laboratory have shown that normal human plasma rendered free from cellular elements, by Berkefeld filtration, is capable of reducing the coagulation time of the blood of patients with hemophilia both *in vitro* and *in vivo* (1). It has also been demonstrated that this coagulation activity of the plasma was associated with the globulin fraction of the plasma proteins (2, 3). The published data do not however identify the active material as a protein, nor do they differentiate it from fibrinogen and prothrombin, constituents of the globulin fraction which also play a role in blood coagulation. Indeed "globulin substance" as prepared by previous methods was known to contain both prothrombin and fibrinogen (2). The present communication concerns a study of the clot promoting activity of normal human plasma, after the removal of prothrombin and fibrinogen.

PREPARATION OF MATERIALS

Several authors (4, 5, 6) have described methods for the removal of prothrombin from blood plasma. These methods consist essentially of adsorption of this substance on the hydroxides of aluminum, calcium, or magnesium. Chew (7) has suggested recently that following filtration through Seitz filters (calcium magnesium aluminum iron silicate) a prothrombin free filtrate of plasma can be obtained.

By the use of the Quick *et al.* technique (8) it is possible to obtain a measure of the amount of prothrombin contained in preparations made from plasma.² Table I

¹ The expenses of this investigation were defrayed in part by a gift to Harvard University from Smith, Kline, and French Laboratories, Philadelphia and by a grant given in honor of Francis Weld Peabody by the Ella Sachs Plotz Foundation.

² All plasmas studied were derived from normal human 0.25 per cent citrated blood centrifuged 30 minutes at 2000 r.p.m., filtered through 2 thicknesses of Number 2 Whatman paper and then through a Berkefeld V filter

shows the prothrombin times of certain of these preparations. It will be observed that while preparations with commercial aluminum hydroxide were quite inefficient the use of aluminum hydroxide C-gamma (9) gave preparations relatively free from prothrombin. The amount of prothrombin remaining depended upon the number of times the adsorption was repeated. More striking results were obtained however by passing Berkefeld filtrates of citrated normal human plasma 5 times through Seitz filters using fresh pads for each filtration. In addition to being prothrombin free these preparations had the advantage of being sterile. On the basis of these observations the Seitz filtration method was used routinely for the removal of prothrombin from plasma. Each batch of plasma treated in this manner was tested for prothrombin by the Quick technique and none was found. The presence of fibrinogen in the Seitz filtrate was demonstrated by the formation of a clot upon the addition of fresh serum as a source of thrombin. In one typical experiment one-tenth ml. of such serum clotted one-tenth ml. of the Seitz filtrate in 29 seconds.

Plasma was rendered free from fibrinogen by heat coagulation. The Berkefeld filtrate from fresh citrated normal human plasma was heated to 56° C. in the water bath. It was held at this temperature for 2 minutes and the copious precipitate containing the fibrinogen was removed by filtration. The absence of fibrinogen in the filtrate was demonstrated by the failure of clot formation when fresh serum acting as a source of thrombin was added.

TABLE I

The effect of certain adsorbents on the removal of prothrombin from normal citrated plasma as measured by the Quick prothrombin time²

Method of preparation of Berkefeld plasma	"Prothrombin time. Average of three determinations"
	seconds
None (control)	29
Commercial aluminum hydroxide 50 cc. plasma + 50 cc. alumina cream	36
Above procedure repeated 3 times	40
Aluminum hydroxide C-gamma (1 gram) to 50 cc. plasma	73
Above procedure repeated twice	217
Above procedure repeated 3 times	510
Five times through Seitz pads	No clot

For the preparation of plasma free from both fibrinogen and prothrombin, the fibrinogen was removed by the above technique and the filtrate passed 5 times through Seitz filters to remove the prothrombin. When any of the preparations were to be stored they were dried by the lyophilic method to avoid chemical change during desiccation.

EXPERIMENTAL

In vitro studies

In vitro determinations of the clot promoting activity of plasma freed from prothrombin or both prothrombin and fibrinogen were made. Varying amounts of the preparations to be tested were added to 2 ml of hemophilic blood, using the standard technique previously described (3). In some instances the results obtained were compared with the Berkefeld plasma from which the preparation was derived. Precise quantitative relationships were not possible since there was a loss of plasma to the filter pads and some evaporation occurred during the time required for Seitz filtration. The results however were sufficiently clear cut to permit conclusions to be drawn. A

summary of the data from typical experiments is given in Table II.

These data indicate that the greater part of the clot promoting activity of cellular free normal human plasma is not dependent on the presence of either fibrinogen or prothrombin, and remains in the filtrate from which these proteins are removed.

The effect, in vivo, of a single injection of normal human plasma free from prothrombin on the coagulation time of the blood of a patient with hemophilia

Two patients with hemophilia served for *in vivo* studies. In one, a single intravenous injection of 50 ml of normal human plasma free from prothrombin was administered. The coagulation time fell from 108 to 28 minutes 1 hour after the injection was given. It remained at this low level for 7 hours, after which it returned slowly to a value of 128 minutes in the course of 69 hours. The results are shown graphically in Figure 1. The observations were repeated on the

TABLE II

The effect of preparations of citrated normal human plasma free from prothrombin and fibrinogen on the coagulation time of hemophilic blood in vitro

Control coagulation time of 2 ml of blood from patient with hemophilia	Preparation employed	Coagulation time of 2 ml of blood from patient with hemophilia after the addition of preparations in the amounts shown below		
		0.01 ml	0.05 ml	0.10 ml
minutes		minutes	minutes	minutes
129	Original Berkefeld plasma			14
129	Original after 5 times through Seitz filter			16
25	Original Berkefeld plasma			10
25	Original after 5 times through Seitz filter			13
36	Filtered 5 times through Seitz filter	23	21	20
42	Fibrinogen free plasma Filtered 5 times through Seitz filter	25	18	17
81	Fibrinogen free plasma Filtered 5 times through Seitz filter		21	16
136	Fibrinogen free plasma Filtered 5 times through Seitz filter	33	26	18
136	Fibrinogen free plasma Filtered 5 times through Seitz filter	59	30	24
35	Fibrinogen free plasma Filtered 5 times through Seitz filter	28	23	18

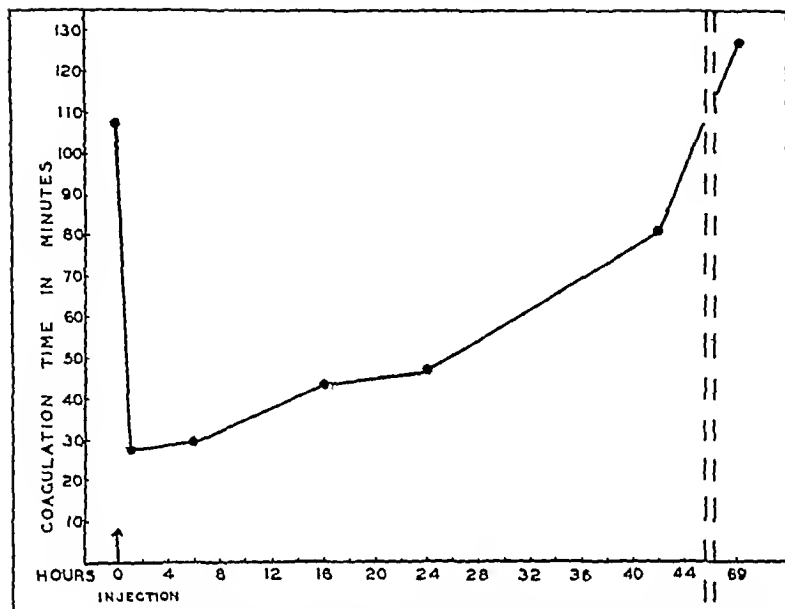


FIG. 1 EFFECT OF A SINGLE INTRAVENOUS INJECTION OF NORMAL HUMAN PLASMA FREE FROM *prothrombin* ON THE BLOOD COAGULATION TIME IN A PATIENT WITH HEMOPHILIA

second patient with hemophilia with entirely similar results

The effect, in vivo, of a single injection of normal human plasma free from fibrinogen and prothrombin on the coagulation time of the blood of a patient with hemophilia

A patient with hemophilia was given a single intravenous injection of 50 ml of normal human plasma free from fibrinogen and prothrombin. The coagulation time of his blood fell from 110 minutes to 26 minutes 1 hour after the injection. This low level persisted for 6 hours after which it slowly returned to the pre injection level reaching 100 minutes in 52 hours after the injection. These results are shown graphically in Figure 2. A similar response to the administration of this material was obtained in a second patient with hemophilia. By a comparison of the data pre-

sented graphically in Figure 1 with the data previously published (1) on single intravenous injections of normal human citrated plasma into patients with hemophilia no essential difference can be discerned. Figures 1 and 2 are virtually superimposable indicating that the removal of fibrinogen in addition to prothrombin did not diminish the effectiveness of the clot promoting activity of the filtrate.

The effect in vivo of multiple injections of normal human plasma free from fibrinogen and prothrombin on the coagulation time of the blood of a patient with hemophilia

A patient with hemophilia was given a total of 4 intravenous injections of 50 ml of normal human plasma free from fibrinogen and prothrombin. The injections were given at intervals of 6 hours. In 1 hour following the initial injection

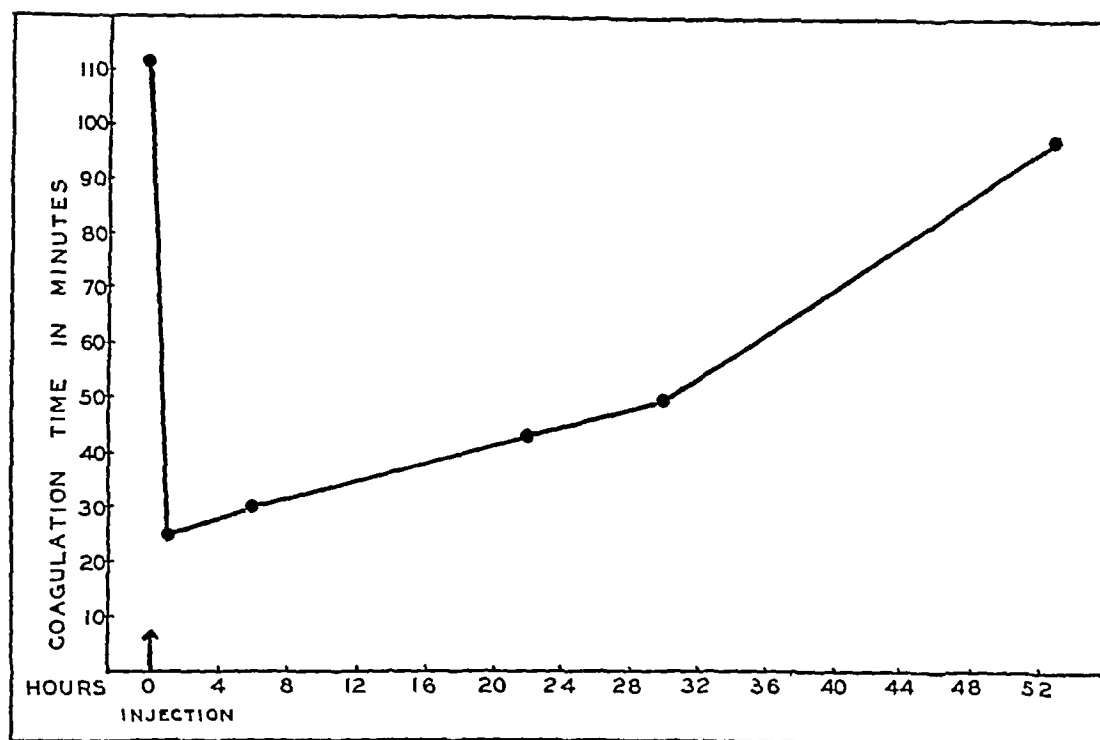


FIG 2 EFFECT OF A SINGLE INTRAVENOUS INJECTION OF NORMAL HUMAN PLASMA FREE FROM *prothrombin* AND *fibrinogen* ON THE BLOOD COAGULATION TIME IN A PATIENT WITH HEMOPHILIA

the coagulation time of the blood of the patient fell from 135 to 40 minutes. It remained between 40 and 45 minutes for the next 6 hours. Following the second injection the coagulation time fell to 20 minutes and remained at a level between 20 and 30 minutes after the subsequent injections. After the fourth and last injection the coagulation time of the blood remained between 30 and 40 minutes for a period of 25 hours after which it slowly returned in 3½ days to pre-injection levels. The data are presented graphically in Figure 3. A comparison of these data with those previously published for multiple injections of unmodified normal human plasma (1) shows a close correspondence. The maintenance of a lowered coagulation time by repeated injections of unmodified plasma and preparations of plasma free from prothrombin and fibrinogen is in sharp contrast to the refractory phase reported as accompanying multiple injections of acid-precipitated "globulin substance" (3).

DISCUSSION

In an earlier publication (2) it was pointed out that against a calcium-fibrinogen system "globu-

lin substance" behaved as prothrombin in the production of a fibrin clot. It was also shown that in this regard the preparations from the cellular free blood plasma of both normal persons and cases of hemophilia behaved in a similar manner. It was quite evident therefore that "globulin substance" contained prothrombin as defined by the clotting mechanism.

The "prothrombin time" as determined by the method of Quick *et al* (8) of patients with hemophilia is normal. A fall of 20 per cent of the total amount of prothrombin may occur, however, without appreciable change in the "prothrombin time" (8). For this reason a normal "prothrombin time" would not necessarily mean that the addition of more prothrombin could not force the coagulation mechanism toward clot formation, and hence decrease the coagulation time of the blood. It becomes necessary therefore to know whether the clot promoting activity of plasma described in earlier investigations published from this laboratory (1, 2) can exist in the absence of prothrombin.

The present observations seem to answer this question. Both in the test tube and following in-

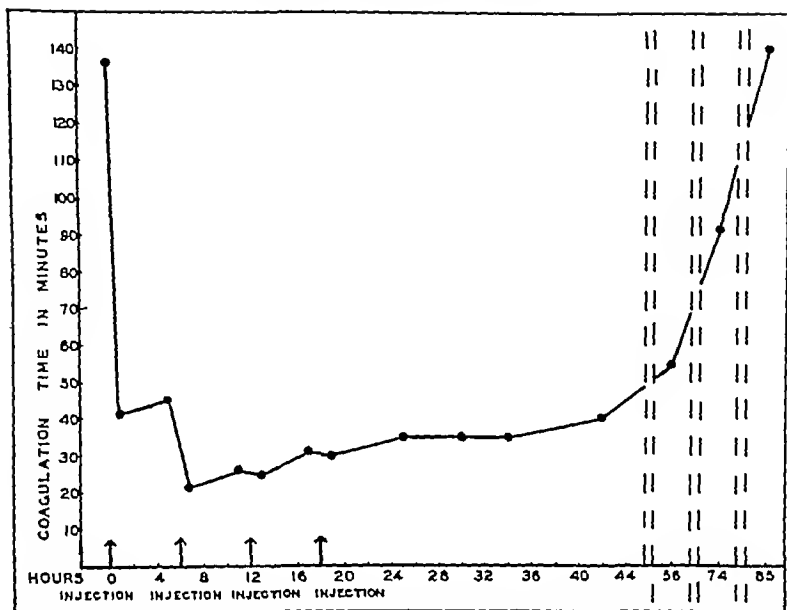


FIG. 3 EFFECT OF MULTIPLE INTRAVENOUS INJECTIONS OF NORMAL HUMAN PLASMA FREE FROM prothrombin and fibrinogen ON THE BLOOD COAGULATION TIME IN A PATIENT WITH HEMOPHILIA

jection into patients with hemophilia the clot promoting activity occurs in plasma which contains no prothrombin. The same conclusions so far as *in vitro* experiments are concerned have been arrived at independently by Frank and Hartmann (6) and by Howell (11) who terms the active factor "plasma thromboplastin".

Recently Macfarlane has reported an individual who had a prolonged coagulation time and in whose blood no fibrinogen could be found (12). Since the presence of fibrinogen in 'globulin substance' has been recorded (2), and since variations within 'normal' limits in the fibrinogen content of the blood in hemophilia are known to occur it is again necessary to differentiate the clot promoting activity from this protein.

The data presented in this paper and also the experiences of Howell show that while fibrinogen is present in euglobulin (2) and in "globulin substance" prepared by acid precipitation, the removal of fibrinogen does not destroy the clot

promoting activity of plasma. The *in vitro* observations of Howell and the data of the *in vitro* and *in vivo* observations of the present communication suggest that so far as the patients studied are concerned, the coagulation defect in hemophilia does not lie in the prothrombin or fibrinogen fractions of the plasma proteins.

It is not implied, however that deficiencies in fibrinogen and prothrombin may not modify the action of normal human plasma or derivatives of it on the blood of certain cases of hemophilia. Indeed Macfarlane's patient with congenital absence of fibrinogen and our own experiences with 2 atypical cases of hemophilia (13) would suggest that certain multiple deficiencies of factors controlling clotting may occur. Such interrelationships between the various substances present could be anticipated in a system of multiple components such as are concerned in the coagulation of the blood. The present report shows only that there exists in cellular free normal human

factor as well, in which the rod phase is delayed (Figure 1 Curve 3). This is illustrated in Figure 1 by the difference between cone rod transition points CR and CR¹ (Curves 2 and 3). In certain instances this time factor, or speed, alone is altered and the final threshold is normal. In others, both the speed and final threshold are abnormal. On this account it is essential to the use of dark adaptation in clinical studies that a procedure be employed which follows the entire course of adaptation rather than one which confines itself to a single determination of the final rod threshold.

Measurements

For the purpose of this study 3 points in the curve are considered to be of especial importance, namely the cone-rod transition point, the 20-minute reading and the 40-minute reading. The transition point gives an indication of the speed of adaptation; the 20-minute reading is the resultant of several factors including speed slope (which is a function of speed) and threshold. If any change in either speed or threshold occurs it is generally discernible at the 20-minute reading. The 40-minute reading is the final value reached with complete dark adaptation. Since normal values fall within a narrow, defined range, an appreciable shift at any of these 3 points is considered to be abnormal.

Control observations on 15 normal subjects are recorded in Table I. The cone-rod transition point always occurred before 13 minutes, the 20-minute value did not exceed a log *I* value of 4.0, the final threshold did not exceed a log *I* value of 3.5. Differences of 0.3 log units are considered significant in the same subject. Such changes correspond to 100 per cent alterations in brightness.

In Table II are recorded observations on 35 patients with various diseases, including rheumatoid arthritis, lobar pneumonia, chronic nephritis, diabetes mellitus, essential hypertension, renal stone, multiple sclerosis. In general, the values agreed with those obtained from normal subjects. Minimal changes occurred in certain of these patients. However, significant changes occurred in several with renal stone. Although less constant these findings confirm in part the observations of Ezickson and Feldman (23). Of 12 cases of renal

TABLE I
Dark adaptation data on 15 normal subjects

Case	Age	Sex	Log threshold (micrometrolamberts)			Cone-rod transition point
			8 minutes	20 minutes	40 minutes	
R. M	30	M	5.8	3.7	3.2	12.1
W. M	30	M	5.6	3.5	3.4	10.6
L. L.	30	F	6.0	3.8	3.2	11.8
C. H.	40	M	6.2	3.9	3.4	12.0
F. K.	40	M	6.0	3.6	3.2	10.0
W. G.	40	M	6.2	3.7	3.5	10.3
D. B.	34	F	6.1	3.9	3.3	12.0
J. P.	27	M	6.0	3.6	3.3	10.6
D. R.	26	F	6.1	4.0	3.3	10.6
E. H.	40	F	6.1	3.8	3.4	12.0
A. P.	34	M	5.7	3.5	3.3	10.4
M. A.	25	F	6.0	3.8	3.0	12.0
J. P.	26	M	5.9	3.7	3.3	9.6
D. S.	40	M	6.1	4.0	3.5	12.0
R. D.	30	M	5.8	3.5	3.0	10.8

TABLE II
Dark adaptation data on patients with various diseases

Case	Age	Sex	Log threshold (micrometrolamberts)			Cone-rod transition point	Diagnosis
			8 minutes	20 minutes	40 minutes		
M. L.	26	F	5.9	3.9	3.4	13.0	Rheumatoid arthritis
H. M.	40	F	5.8	3.8	3.0	13.0	Rheumatoid arthritis
E. M.	38	F	6.0	3.7	3.2	11.0	Rheumatoid arthritis
H. T.	31	M	5.9	3.7	3.4	8.3	Rheumatoid arthritis
M. H.	42	F	6.0	4.0	3.4	13.7	Renal stone
A. G.	44	F	6.1	4.0	3.2	11.0	Renal stone
A. J.	28	F	5.9	3.4	3.3	9.5	Renal stone
M. L.	63	F	6.5	4.0	3.6	13.0	Renal stone
S. L.	31	M	6.0	3.8	3.1	11.0	Renal stone
A. C.	54	F	6.3	4.6	3.5	14.8	Renal stone
A. L.	50	M	6.4	4.0	3.8	9.4	Renal stone
G. O.	28	M	6.0	3.8	3.7	10.4	Renal stone
J. C.	59	M	6.6	4.1	3.9	10.8	Renal stone
R. R.	46	M	6.2	4.0	3.1	9.4	Renal stone
E. R.	37	F	6.5	3.9	3.4	10.0	Renal stone
T. R.	72	M	6.6	3.9	3.9	18.7	Renal stone
M. M.	34	M	5.8	3.8	3.2	11.5	Renal stone
M. G.	35	M	5.8	3.5	3.5	9.4	Lobar pneumonia
J. S.	24	M	5.9	3.5	3.2	10.0	Lobar pneumonia
K. D.	20	F	5.8	3.6	3.2	12.5	Lobar pneumonia
J. C.	63	M	6.2	4.1	3.4	12.8	Chronic nephritis
R. N.	38	F	6.0	4.2	3.4	12.5	Essential hypertension
R. B.	48	F	6.2	3.6	3.5	11.5	Essential hypertension
A. R.	36	M	6.2	4.0	3.3	12.5	Chronic nephritis
S. M.	25	M	5.8	3.5	3.7	9.5	Chronic nephritis
H. H.	39	F	5.6	3.5	3.0	10.0	Diabetes mellitus
F. F.	58	F	6.0	4.0	3.5	9.0	Diabetes mellitus
R. S.	39	F	6.2	3.7	3.2	11.0	Diabetes mellitus
J. P.	25	F	6.0	4.0	3.5	13.2	Multiple sclerosis
D. F.	28	M	6.2	4.4	3.4	12.0	Multiple sclerosis
J. C.	25	M	5.7	3.7	3.2	11.5	Multiple sclerosis
B. A.	30	M	6.0	4.2	3.4	12.5	Multiple sclerosis
P. T.	30	M	6.0	3.8	3.5	11.2	Multiple sclerosis
P. E.	40	M	6.7	4.4	4.0	11.0	Multiple sclerosis
W. W.	35	M	6.9	4.0	3.4	10.6	Multiple sclerosis

stone, 4 showed slight elevation of final threshold. 1 showed slight delay of rod function, while 3 showed marked delay of rod function, quite like

tive relation between available vitamin A and the regeneration of visual purple in the retinal rods during dark adaptation. A number of recent studies (15, 16, 17) have shown this relationship to exist. The particular instrument used (18) allows examination of the full course of dark adaptation after exposure to a preadapting light. The curves are precise and reproducible, the physiological day-to-day variation being greater than the experimental error. The procedure is controlled with regard to brightness, duration, color and retinal location of the preadapting light as well as the ensuing dark adaptation of a sharply defined retinal area. The white preadapting brightness of 4700 millilamberts¹ is viewed by the subject for 4 minutes. The test light, a flash of 0.2 second's duration, passes through a violet filter (Corning 511) which transmits the spectrum only below 460 mμ. The retinal region tested is a circular area whose diameter subtends 3° visual angle and is located 7° nasally in the right eye. No artificial pupil was used, as it has been demonstrated that the pupillary reflex error in rod measurements is clinically negligible under the conditions of the present experiments (19).

¹ Millilamberts = foot candles $\times 1.0764$, micromicrolamberts = millilamberts $\times 10^9$

Types of dark adaptation

In Figure 1 is illustrated the course of dark adaptation of 15 normal subjects (shaded area 1) by a plot of log threshold brightness in micromicrolamberts¹ as a function of time in the dark. After a 4-minute exposure to white light 4700 millilamberts, the first phase (a) of cone adaptation takes place, to be followed by the rod phase (b). If the normal person is subjected to a diet low in vitamin A for a sufficient length of time, the final threshold becomes raised not only for the rods but also for the cones (20, 21, 22). The cone changes were noted originally in cases of liver cirrhosis (14). A similar curve of "simple deficiency" is seen in the patient with obstructive jaundice (Figure 1, Curve 2), whose night blindness is related to malabsorption of vitamin A. However, in patients with cirrhosis of the liver, there may be not only a raised final threshold indicative of vitamin A deficiency, but a time

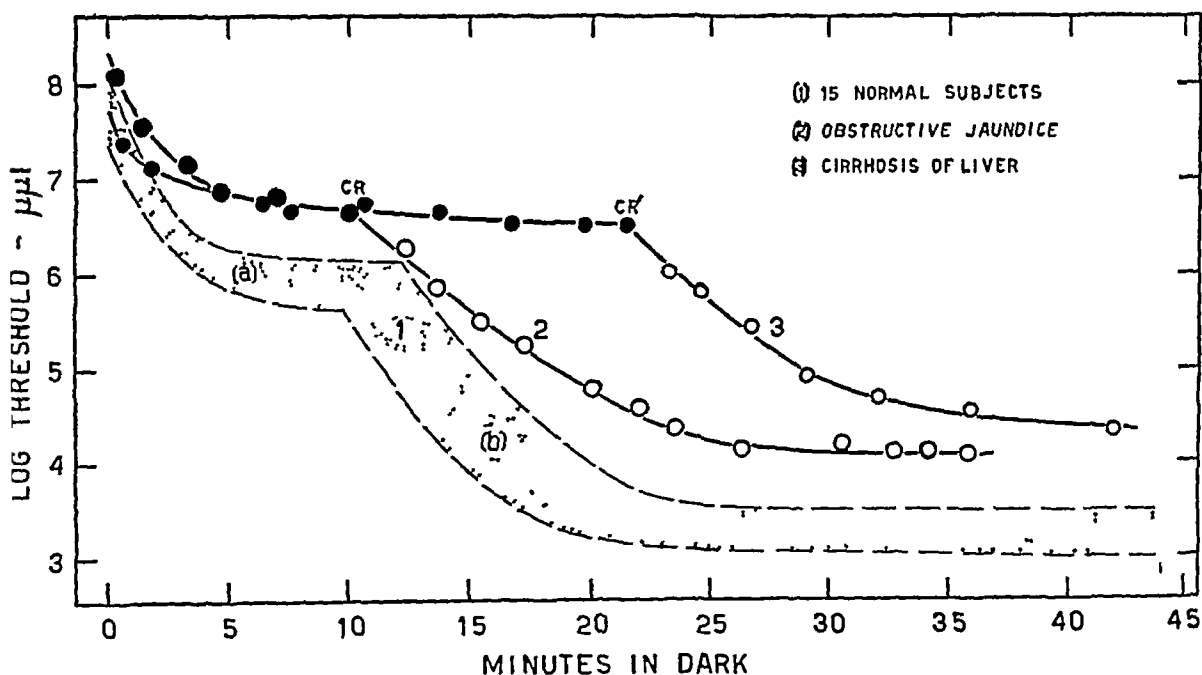


FIG. 1. NORMAL AND ABNORMAL DARK ADAPTATION CURVES

The log threshold brightness is expressed in micromicrolamberts (millilamberts $\times 10^9$). The points indicate single observations. Those which appear violet to the subject fall on the cone portion of the curve and are denoted by solid symbols. Those which appear colorless to the subject fall on the rod portion of the curve and are denoted by open symbols.

The shaded area (1) represents the range of variation in 15 normal subjects as well as the extent of extreme daily variation. Curve 2 is that of a patient with obstructive jaundice. This corresponds in shape to the curve of "simple" vitamin A deficiency. In Curve 3, from a patient with cirrhosis of the liver, the rod-cone transition point is displaced and the final thresholds for both cones and rods are elevated.

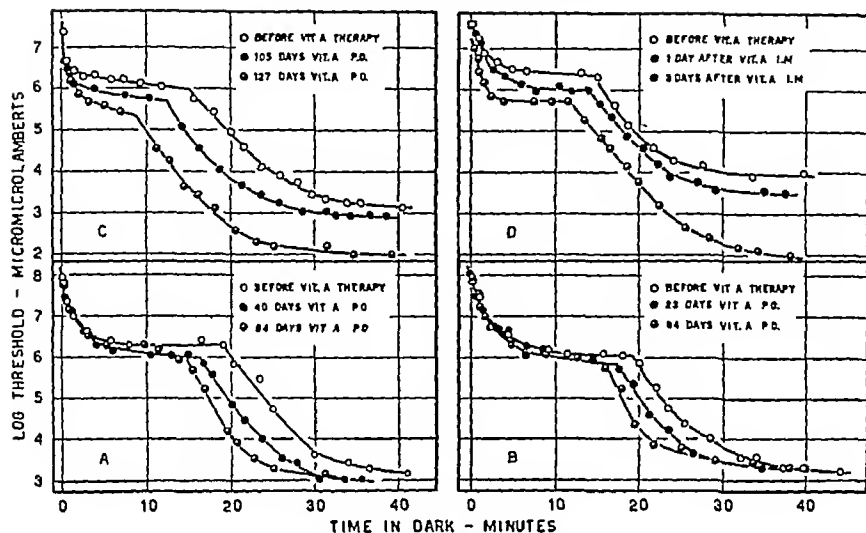


FIG. 2. CHANGES IN DARK ADAPTATION OF 4 PATIENTS WITH CIRRHOSIS OF THE LIVER DURING VITAMIN A THERAPY

The three curves drawn for each case are selected from actual readings made during the course of vitamin A therapy. The upper curves from Cases C and D reveal high cone and rod final thresholds together with delay in rod adaptation. Following vitamin A therapy both of these functions improved. The procedure for measuring dark adaptation in Case C was somewhat different from the standard technique employed in the other cases (14).

The lower curves from Cases A and B reveal normal final thresholds but abnormal delay at the cone rod transition point. In patients with cirrhosis of the liver this type of change appears to be the more characteristic. These curves also show the improvement that follows vitamin A therapy.

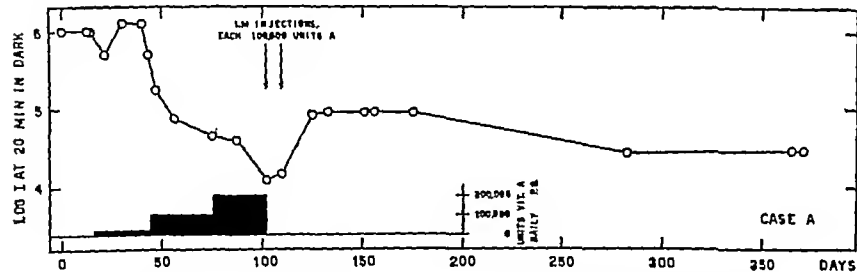


FIG. 3. CHANGES IN THE 20-MINUTE DARK ADAPTATION THRESHOLD WITH VITAMIN A THERAPY

The patient (Case A) was fed 10,000 LU vitamin A concentrate daily for 1 month without apparent benefit. This period was followed by 1 month with 100,000 LU daily during which a significant improvement in dark adaptation was observed. The dose was then increased to 200,000 units daily during which improvement continued. After 2 weeks of such therapy the patient received 2 injections intramuscularly of 100,000 units which caused no further change. On discontinuing treatment there followed a rise and subsequent decline of threshold. Partial improvement was maintained without specific therapy for 9 months.

the alterations seen in patients with cirrhosis of the liver

Although the age and sex distribution of the above 2 groups differs from that of our patients with cirrhosis of the liver, earlier studies have indicated these differences to be of little, if any, significance

Observations on 24 cases of cirrhosis are recorded in Table III. Fifteen showed delay at the

TABLE III

Dark adaptation data on patients with cirrhosis of the liver

Case	Age	Sex	Log threshold (micromicrolamberts)			Cone-rod transition point
			8 minutes	20 minutes	40 minutes	
J. H.	years 60	M	6.3	6.3	3.3	minutes 21.5
J. C.	63	M	6.3	5.6	3.9	16.8
W. M.	55	M	6.2	6.0	4.0	19.5
B. M.	40	M	6.3	6.0	3.3	19.0
A. L.	57	M	6.2	5.9	3.3	18.0
R. C.	46	M	6.5	5.4	4.2	15.5
C. J.	64	M	7.1	6.9	5.0	19.0
R. T.	52	M	6.0	4.4	3.9	15.5
E. W.	41	F	6.2	4.6	3.4	15.3
V. T.	49	M	6.2	4.9	3.5	15.0
J. K.	48	M	6.3	5.0	4.3	15.5
M. F.	45	F	6.1	4.4	3.4	14.5
J. H.	61	M	6.5	4.8	4.0	13.3
J. L.	60	M	6.2	4.4	4.0	11.6
A. LeC.	58	M	6.5	5.2	4.3	11.8
M. M.	49	F	7.0	4.6	4.1	13.3
F. S.	53	M	6.3	4.4	4.0	13.0
E. G.	45	F	6.9	4.0	3.7	11.9
W. H.	38	M	7.0	3.9	3.7	10.3
R. B.	60	M	6.0	4.3	3.5	10.5
M. DeF.	49	F	6.4	4.0	3.4	12.4
E. A.	45	F	6.0	4.0	3.7	12.0
M. O.	38	F	6.2	5.9	3.4	19.8
P. K.	45	M	6.0	3.8	3.2	11.8

transition point, 19 showed high readings at 20 minutes, 11 showed elevated final rod thresholds. Of 6 cases with almost normal readings, 4 had received vitamin A concentrates for from 3 to 12 months prior to testing. There was no apparent correlation between the severity of liver disease and the degree of derangement in dark adaptation.

Response to therapy

After preliminary tests were made, the patients with cirrhosis of the liver were fed a diet rich in meat, green vegetables, milk, fruit, and eggs. By calculation from food tables (24) it was estimated to provide at least 13,000 I.U. Vitamin A

daily. It also contained supplements of Vitamin "B complex" and ascorbic acid, in the form of thiamin chloride, brewer's yeast, liver extract, and orange juice. On this regimen 2 patients showed moderate improvement in speed of adaptation after 7 months. However in 5 other cases, similarly treated, no changes occurred after intervals varying between 6 and 18 months.

Concentrates of vitamin A² were then administered to several patients orally and parenterally in addition to the above diet. Detailed observations illustrating dosages used and the response to such therapy in 4 patients are shown in Figure 2. The upper curves, from Cases C and D, reveal delayed cone-rod transition points and high rod and cone thresholds. Both of these functions improved strikingly after therapy. The lower curves, from Cases A and B, reveal marked delay at the transition point but no elevation in final thresholds of either rods or cones. These curves likewise reveal the return towards normal values after therapy. It is noteworthy that changes of rod function are confined almost entirely to alterations in speed.

Examination of the curves of Case D (Figure 2) reveals marked increases in the slope or speed of the cone function which parallel increments in the speed of the rod function. This subject had a pupil which was fixed with respect to light stimuli at a diameter of about 6 mm, whereas the other subjects, who showed no measurable change in cone speed, had normal pupillary light reflexes. Comparable improvements in the speed of the cone functions of Cases A, B, and C might have been detectable if a constant pupillary aperture had been employed in making the measurements. Just such a discrepancy between measurements made with and without a constant pupillary aperture are predictable from recent experiments (19) on the effect of the pupillary light reflex upon cone dark adaptation measurements.

In order to show the changes that occurred over several months' observations, the 20-minute rod readings were selected for comparison in these cases (*cf* Figures 3, 4, 5, 6). In Cases A

² A concentrate of the National Oil Products Co., Newark, N. J., was diluted with peanut oil to make appropriate doses. Oil for parenteral use was autoclaved in air-tight bottles.

THE VARIABILITY OF PROTEINURIA IN THE HYPERTENSIVE COMPLICATIONS OF PREGNANCY

By LEON C. CHESLEY

(From the Department of Biochemistry Margaret Hague Maternity Hospital Jersey City)

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Proteinuria in eclampsia was first described by Lever (1) in 1843 and since that time has been interpreted by many writers as a sign of a renal lesion underlying the toxemias of pregnancy. In late years however arteriolar, and possibly capillary spasms have been advanced as an explanation for the leakage of protein through the glomerular capillaries, not only in the toxemias of pregnancy but also in acute glomerulonephritis and in essential hypertension (Volhard (2) Irving (3) Eastman (4)). In a recent paper Chesley Markowitz and Wetchler (5) have shown that even very transitory vasospasm does result in proteinuria.

Perhaps a study of the variability of protein leakage into the urine during successive periods would yield data pointing toward one of the other alternative interpretations of proteinuria in toxemia. In the presence of a diffuse renal lesion one might expect a nearly constant leakage of protein. On the other hand if the proteinuria were caused by vascular constriction the protein leakage might fluctuate from moment to moment, waxing and waning with more and less intense vascular spasms. If the periods of observation be too long it is conceivable that the proteinuria might 'average out'. As the first approach to the problem the urine output has been collected at hourly intervals. The plasma clearance of endogenous creatinine has been determined as a rough measure of the glomerular filtration and compared with the protein excretion. This comparison perhaps enables one to calculate the approximate concentration of protein in the glomerular filtrate (see below) in other words, the calculation gives the protein leakage through the glomerular capillaries.

MATERIAL AND METHODS

Patients were selected in whom the differential diagnosis could be made between preeclamptic toxemia and Bright's disease complicated by pregnancy. The criteria for preeclamptic toxemia in-

clude a rapid and excessive gain in weight hypertension proteinuria, and edema all appearing for the first time late in the pregnancy—usually in the order mentioned—and disappearing rapidly following delivery. Renal function is normal insofar as it is shown by the urea clearance. Nearly all patients used were primigravidae. The criteria for Bright's disease are hypertension, proteinuria edema hematuria and persistently lowered urea clearance. In nearly all cases used in this study some of these signs were known to have existed either before the pregnancy or early in the pregnancy. Follow up of these patients proved these signs to be persistent. Subjects in both groups were used, as were a few non pregnant nephritics from the Jersey City Medical Center. In almost all cases the patients were *antepartum* and near term. Two of the pregnant nephritics were in their fourth and sixth months of gestation. Four urine specimens were collected by catheter at hourly intervals. Midway in the test period blood was taken for a plasma creatinine determination.

The urine was centrifuged at 1500 r.p.m. for ten minutes and the clear supernatant fluid was used in all analyses.

Urinary protein. Duplicate samples of from 0.5 to 5 ml of urine depending upon the degree of proteinuria were diluted to about 10 ml in centrifuge tubes. Two ml each of 10 per cent sodium tungstate and $\frac{3}{4}$ N sulfuric acid were then added. After precipitating overnight the protein was thrown down by centrifugation the supernatant fluid decanted and the protein redissolved in 1 ml of 10 per cent sodium tungstate. After dilution to about 14 ml, 1 ml of $\frac{3}{4}$ N sulfuric acid was added. The following day the precipitates were again centrifuged down dissolved in dilute sodium hydroxide rinsed into NPN tubes and digested with sulfuric acid and hydrogen peroxide the solutions were nesslerized and read against nitrogen standards of comparable color.

There remains the implication that in addition to the above possible causes for vitamin A deficiency, the injured liver alters the intermediary metabolism of vitamin A. This is supported by the observation that certain cases show only delay of response but ultimately reach normal thresholds. In other words, the reserve for visual purple and hence vitamin A was sufficient to allow a normal threshold to be reached, but the regeneration of visual purple was impeded.

SUMMARY AND CONCLUSIONS

1 Abnormal dark adaptation was observed in 19 of 24 patients with cirrhosis of the liver.

2 In certain patients both elevation of the final rod and cone thresholds and delay of rod dark adaptation occurred. In most instances only the latter change took place.

3 These changes were unrelated to jaundice. They tended to persist in the presence of a nutritious diet, rich in vitamin A.

4 The administration orally and parenterally of vitamin A concentrates was followed by extensive improvement. Cone and rod thresholds were lowered, and speed of adaptation was increased. After discontinuance of therapy this improvement was only partially maintained.

5 These findings suggest that abnormal dark adaptation in patients with cirrhosis of the liver is due chiefly to altered intermediary metabolism of vitamin A.

BIBLIOGRAPHY

- 1 Wilbur, D. L., and Eusterman, G. B., Nutritional night blindness. Report of a case. *J. A. M. A.*, 1934, 102, 364.
- 2 Jeghers, H., Night blindness as a criterion of vitamin A deficiency. *Ann. Int. Med.*, 1937, 10, 1304.
- 3 Altschule, M. D., Vitamin A deficiency in spite of adequate diet in congenital atresia of bile ducts and jaundice. *Arch. Path.*, 1935, 20, 845.
- 4 Lasch, F., Über den Vitamin A—Spiegel im Blute bei Leberkrankheiten. *Klin. Wchnschr.*, 1938, 17, 1107.
- 5 Breusch, F., and Scalabrino, R., Die quantitativen Verhältnisse der Leberlipide. *Ztschr. f. d. ges. exper. med.*, 1934, 94, 569.
- 6 Moore, T., The vitamin A reserve of the adult human being in health and disease. *Biochem. J.*, 1937, 31, 155.
- 7 Kumagai, N., Zur Kenntnis der Bewegungsvorgänge in der Netzhaut. *Mitt. a.d. Med. Fakult. d. k. Univ. zu Tokyo*, 1916, 16, 137.
- 8 Ralli, E. P., Pariente, A., Flaum, G., and Waterhouse, A., A study of vitamin A deficiency in normal and depancreatized dogs. *Am. J. Physiol.*, 1933, 103, 458.
- 9 Lasch, F., Vitamin A—Stoffwechsel und Leber Bei Experimenteller Phosphorvergiftung. *Klin. Wchnschr.*, 1935, Nr. 30, 1070.
- 10 Greaves, J. D., and Schmidt, C. L., The utilization of carotene by jaundiced and phosphorous treated vitamin A deficient rats. *Am. J. Physiol.*, 1935, 111, 502.
- 11 Althof, H., and Müller, H., Störungen des Sehvermögens neben solchen der Leberthätigkeit. *Würzb. Med. Ztschr.*, 1861, 2, 349.
- 12 Dolganoff, W., Ueber die Veränderungen des Auges nach Ligatur der Gallenblase. *Arch. f. Augenh.*, 1897, 34, 196.
- 13 Greaves, J. D., and Schmidt, C. L., On the absorption and utilization of carotene and vitamin A in choledochocolonostomized vitamin A deficient rats. *Am. J. Physiol.*, 1935, 111, 492.
- 14 Haig, C., Hecht, S., and Patek, A. J., Jr., Vitamin A and rod-cone dark adaptation in cirrhosis of the liver. *Science*, 1938, 87, 534.
- 15 Fridericia, L. S., and Holm, E., Experimental contribution to the study of the relation between night blindness and malnutrition. *Am. J. Physiol.*, 1925, 73, 63.
- 16 Tansley, K., The regeneration of visual purple its relation to dark adaptation and night blindness. *J. Physiol.*, 1931, 71, 442.
- 17 Wald, G., Carotenoids and the visual cycle. *J. Gen. Physiol.*, 1935, 19, 351.
- 18 Hecht, S., and Schlaer, S., An adaptometer for measuring human dark adaptation. *J. Optic. Soc. America*, 1938, 28, 269.
- 19 Haig, C., The influence of the pupillary light reflex upon dark adaptation measurements. *Anat. Rec.*, 1938, 72, suppl., 82.
- 20 Hecht, S., and Mandelbaum, J., Rod-cone dark adaptation and vitamin A. *Science*, 1938, 88, 219.
- 21 Wald, G., Jeghers, H., and Arminio, J., An experiment in human dietary night blindness. *Am. J. Physiol.*, 1938, 123, 732.
- 22 Booher, L. E., Callison, E. C., and Hewston, E. M., An experimental determination of the minimum vitamin A requirements of normal adults. *J. Nutrition*, 1939, 17, 317.
- 23 Ezickson, W. J., and Feldman, J. B., Signs of vitamin A deficiency in the eye correlated with urinary lithuasis. *J. A. M. A.*, 1937, 109, 1706.
- 24 Daniel, E. P., and Munsell, H. E., Vitamin Content of Foods. *U. S. Dept. Agric. misc. publ.* 275, June 1937.

The greatest variation occurred in a young boy with rapidly progressing chronic nephritis and hypertensive encephalopathy, in such a case vascular disturbances may well have been present.

This relative constancy of protein filtration in Bright's disease has been previously shown by Hanns (11) who found a parallelism between proteinuria and freezing point depression in urine samples taken at intervals during the day. Bing (12) was perhaps the first to show the parallelism between creatinine excretion and protein excretion and to calculate the (approximate) concentration of protein in the glomerular filtrate. He found in renal disease only slight variation in the protein filtration over very short periods of time (8- to 12 minute intervals). Bing presents further confirmatory studies in a later publication (9), which also show a constancy in protein filtration from day to day.

The findings in 6 pregnant nephritics (8 determinations) are presented in Table I. As in non-pregnant nephritics the protein filtration was nearly the same from hour to hour. In one case the test was repeated after 2 weeks and in another case after 5 months (patients still pregnant). In both the protein concentration in the glomerular filtrate had not changed much in the interval.

In contrast to the nearly constant protein filtration in each individual in the two groups of nephritics, there is marked variation from hour to hour among the eclamptic and preeclamptic patients. In one eclamptic the protein concentration in the glomerular filtrate varied from 0.27 to 1.60 mgm per hundred ml. (492 per cent variation). In one hypertensive toxemia patient with markedly labile blood pressure the protein filtration varied by 300 per cent. The least variation—29 per cent—was found in a patient with mild preeclamptic toxemia. Most of the patients showed variations from 30 to 80 per cent, with a few running higher. The findings in 18 tests are summarized in Table I.

The fluctuating proteinuria shown quantitatively by these studies is a matter of common clinical observation. Many patients with toxemia of pregnancy show markedly variable urinary protein concentrations from morning to morning. While the level of diuresis accounts for much of this, it cannot explain a faint trace on one day, a

4 plus on the next, and perhaps a 1 plus on the third day.

Possibly the determination of the protein filtration, in successive hours, will prove to have some value in differentiating between so-called specific toxemia of pregnancy and Bright's disease in cases where the clinical diagnosis is equivocal. In the present study, care has been taken to select patients in whom the differential diagnosis could be made with some confidence. Further studies of doubtful cases, with diagnosis made by follow up, are in progress. Quite probably some pregnant nephritics will also have toxemia of pregnancy, in such cases the proteinuria would be likely to fluctuate somewhat.

One case of rapidly progressing malignant nephrosclerosis was studied in the fourth month of pregnancy and after abortion (Table I). As the urea clearance fell from 32 per cent to 1.3 per cent, the concentration of protein in the glomerular filtrate increased greatly, from 4.59 to 68.5 mgm per cent.

Finally it might be emphasized that the proteinuria of eclampsia and preeclampsia is quite variable. This variability might be interpreted to mean that an important causative factor for the proteinuria is functional rather than anatomic. The functional changes are probably vascular spasms, as suggested in the introduction above. This was the hypothesis upon which this study was undertaken.

SUMMARY AND CONCLUSIONS

It is assumed that dividing the concentration of urinary protein by the ratio of urinary creatinine to plasma-endogenous creatinine will give the concentration of protein in the glomerular filtrate, or a value proportional to it.

Four urine specimens collected at intervals of an hour, were taken from 6 pregnant and 5 non-pregnant nephritics and from 4 eclamptic and 9 preeclamptic patients. The "protein filtration" was calculated and the variability from hour to hour was determined.

In nephritics, pregnant or not, the protein filtration shows very little variation from hour to hour. In toxemia of pregnancy, the protein filtration is variable. It is suggested that this ~~is~~ a functional cause (vascular spasms) in proteinuria.

Plasma creatinine The method of Folin and Wu (6) was used for the determination of plasma creatinine

Urinary creatinine Folin's method was used for urinary creatinine. All determinations were done in duplicate. Aliquots of urine were chosen which would give close colorimetric matches with the standard.

Calculations The concentration of urine protein has been divided by the concentration ratio of plasma creatinine to urine creatinine. If the plasma clearance of endogenous creatinine measures the glomerular filtration (7) this computation gives the level of protein in the glomerular filtrate. If not quite all of the "apparent creatinine" of the plasma is creatinine, or if there is some tubular secretion of endogenous creatinine, the calculation gives a figure proportional to the protein content of the glomerular filtrate. If the secretion (if any) of creatinine does not vary with filtration, the calculated filtration of protein will vary independently of the actual protein leakage, *i.e.*, the calculation of protein filtration by this means would not be valid. It will be assumed that the calculation is valid. The same assumption has been made for similar calculations by Bing (8) and by Berglund, Scriver and Medes (9) for exogenous creatinine which does appear to be partially secreted by the tubule cells (10). Whether the assumption be valid or not, results were obtained in specific toxemia differing from those found in Bright's disease.

RESULTS AND DISCUSSION

The reliability of the analytical methods was checked repeatedly by doing quadruplicate analyses of a urine for creatinine and protein. The lowest protein value was divided by the highest creatinine, and the highest protein by the lowest creatinine. From the results of this check, it was found that the average analytical error for the calculation of the "protein filtration" was about 5 per cent, in one instance the error was as high as 12 per cent.

To test the validity of the hypothesis that a diffuse renal lesion should result in a nearly constant leakage of protein, demonstrated by measurement of the protein filtration, a control series of non-pregnant nephritic patients has been studied.

TABLE I
Protein filtration in pregnant and non pregnant nephritics, and in eclampsia and preeclamptic toxemia

Diagnosis	Protein in glomerular filtrate*		Maximal variation
	Lowest	Highest	
	mgm per cent	mgm per cent	per cent
NON-PREGNANT NEPHRITICS			
Chronic glomerulo-nephritis	6.64	7.10	7
Nephrotic syndrome	9.95	11.71	18
Chronic glomerulo-nephritis with hypertensive encephalopathy	1.96	2.36	19
Ditto (same patient 2 weeks later)	31.5	38.9	24
Chronic glomerulo-nephritis	2.53	2.81	12
Chronic glomerulo-nephritis	0.144	0.153	6
PREGNANT NEPHRITICS			
Nephrotic syndrome	15.60	18.50	18
Chronic glomerulo nephritis	8.46	8.83	4
Chronic glomerulo-nephritis	7.98	9.20	15
Chronic glomerulo-nephritis	8.59	9.71	13
Ditto (same patient 5 months later)	10.4	12.4	19
Benign nephrosclerosis (?)	3.16	3.59	13
Ditto (same patient 2 weeks later)	3.49	4.05	16
Pyelonephritis	0.93	1.00	8
TOXEMIA OF PREGNANCY			
Eclampsia	4.17	5.52	32
Eclampsia	2.22	2.94	32
Ditto (same patient 2 days later)	3.98	5.96	50
Eclampsia	0.27	1.60	492
Eclampsia	10.95	14.40	32
Severe preeclampsia	1.05	1.57	50
Severe preeclampsia	3.50	5.60	60
Severe preeclampsia	1.56	2.69	73
Mild preeclampsia	4.52	5.73	29
Mild preeclampsia	1.10	1.93	76
Mild preeclampsia	5.42	8.16	51
Mild preeclampsia	2.37	3.48	47
Mild preeclampsia	4.18	7.45	78
Mild preeclampsia	6.40	8.64	35
Hypertension	0.77	1.04	36
Hypertension, blood pressure markedly variable	0.09	0.34	303
Unclassified	2.95	8.59	191
Unclassified	1.65	2.40	45
PREGNANT, MALIGNANT NEPHROSCLEROSIS			
Malignant nephrosclerosis	4.59	5.62	23
Ditto (same patient 4 weeks later)	5.72	6.37	11
Ditto (same patient 10 days later)	31.60	68.50	117

* See text for qualifications and reservations

As Table I shows, the variation in protein filtered from hour to hour is not very great. In 3 of the 5 cases it does not exceed the analytical error.

CLINICAL STUDIES OF THE BLOOD VOLUME VIII MACROCYTIC AND HYPOCHROMIC ANEMIAS DUE TO CHRONIC BLOOD LOSS, HEMOLYSIS AND MISCELLANEOUS CAUSES, AND POLYCYTHEMIA VERA¹

By JOHN G GIBSON 2d ALFRED W HARRIS AND VERNE W SWIGERT

(From the Medical Clinic of the Peter Bent Brigham Hospital and the Department of Medicine Harvard Medical School and the Robert Dutton Evans Memorial for Clinical Research and Preventive Medicine Boston)

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Early studies of the blood volume in secondary anemia leave considerable doubt concerning the actual state of the blood volume and the relation thereof to the severity of the anemia Keith Rowntree and Geraghty (1) concluded from the results obtained with the original dye method that 'considerable variation exists in the total blood volume and plasma volume in cases of secondary anemia. Subsequent limited studies utilizing modifications of both the carbon monoxide and the dye method have found plasma increased (1 2, 3 and 4) normal or not increased (1 5 6 and 7) Total blood volume was considered as increased despite decreased red cell volume (2 3 and 4), within normal limits (1) or decreased (3, 4 5 6 and 7) In chlorosis total blood volume was found to be high (3 and 8) or decreased (2)

This confusing state of affairs arose probably as much from a failure to classify anemias etiologically as from the discrepancies in accepted normal standards of the widely varying and none too reliable techniques employed None of the above authors could find any relationship between the abnormality of blood volume and the severity of the anemia However Robertson and Bock (9) found a markedly decreased total blood volume in soldiers after hemorrhage, frequently as low as 60 per cent of normal They stated that after a certain point the reduction in volume seemed to parallel the decrease in blood pressure. At this stage they thought the blood volume was not restored by body fluids, since such replacement would but further dilute the blood and lower hemoglobin concentration In this critical condition any increase in total blood volume was considered

to be due to an increase in red cells only Bock (10) stated that in chronic anemia "the blood volume is diminished in direct proportion to the decrease in corpuscles" Griesbach (3) stated that in 'healed cases of secondary anemia the blood volume was parallel to the state of healing The most thorough study of the relationship of blood volume to anemia is that of Bennett *et al* (11) in 1938 In their opinion "the estimation of blood volume is a genuine criterion of the severity (of anemia) as indicated by the fact that the fatal cases comprised a group in whom the blood volume was notably reduced"

The subject of blood volume in polycythemia vera has received a great deal of attention all workers agreeing that the total blood volume is tremendously increased (2 10 and 12 to 19 inclusive) due almost entirely to a great volume of circulating red cells, the plasma volume being either normal or slightly increased (10) or decreased (14 and 15) Haden (19) considered that the red cell count gave too low an index of the total increase in red cells but thought that the red cell mass per kilogram of body weight was the most sensitive indicator of the changes in the red cells

Since we have found a striking relationship between the severity of anemia and the level of plasma circulating red cell and total blood volume in pernicious anemia (20) and in Bright's disease (21), it seemed worth while to make a similar study of secondary anemia The main interest of such a study is to correlate the blood volume level with the clinical course and in light of these findings to evaluate from a clinical standpoint the common laboratory criteria of anemia the red cell count hemoglobin and hematocrit It also seemed of interest to make similar studies in cases of polycythemia vera for comparison with results f¹

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BIBLIOGRAPHY

- 1 Lever, J C W, Cases of puerperal convulsions with remarks Guy's Hosp Repts, 1842-1843, series 2, 1, 495
- 2 Volhard, F, Nieren und Ableitende Harnwege. VI Die Albuminurie. Handbuch der inneren Medizin, Julius Springer, Berlin, 1931, 6, part I, 813
- 3 Irving, F C, The vascular aspect of eclampsia. Am. J Obst & Gynec., 1936, 31, 466
- 4 Eastman, N J, The vascular factor in toxemias of late pregnancy Am J Obst. & Gynec., 1937, 34, 549
- 5 Chesley, L C, Markowitz, I, and Wetchler, B B, Proteinuria following momentary vascular constriction. J Clin Invest., 1939, 18, 51
- 6 Peters, J P, and Van Slyke, D D, Quantitative Clinical Chemistry Vol II Methods Williams and Wilkins Co, Baltimore, 1932, pp 602 and 604
- 7 Miller, B F, and Winkler, A W, The renal excretion of endogenous creatinine in man, comparison with exogenous creatinine and inulin. J Clin. Invest., 1938, 17, 31
- 8 Bing, J, Studies on proteinuria Levin and Munksgaard Ejnar Munksgaard, Copenhagen, 1936
- 9 Berglund, H, Scliver, W de M, and Medes, G, Proteinuria and plasma proteins The Kidney in Health and Disease Lea and Febiger, Philadelphia, 1935, pp 508 to 515
- 10 Smith, H W, The Physiology of the Kidney Oxford University Press, New York, 1937
- 11 Hanns, A., L'elimination de l'albumine dans la néphrite chronique. Presse Med, 1922, 30, 1110
- 12 Bing, J, Undersøgelser over albuminuriens mekanisme. Bibliot. f Laeger, 1932, 124, 415

TABLE I

Findings in secondary anemia

Case number	Date	Sex	Age	Height	Weight	Red cell count	Hemo- globin	Hemo- crit	Ve. pres- sure	Cir- cula- tion time	Plas- ma volume	Per centage plasma volume from normal	Red cell volume	Per centage plasma volume from normal	Total blood volume	Per centage plasma volume from normal	Remarks
			years	cm.	kgm.	mill. per 100 cc. men's	grams per 100 cc. men's	per cent	mm. H ₂ O	sec. ends	cc.	per cent	cc.	per cent	cc.	per cent	
20 CASES OF HYPOCHROMIC ANEMIA DUE TO BLOOD LOSS																	
43	June 20 1935	M	26	152.4	40.8	4,600	10.35	28.0	65	17	2525	+16.9	990	-31.3	3515	-2.4	Hemorrhage
167	April 8, 1936	F	31	152.0	37.6	2,450	8.70	25.9	95	11	1855	+14.1	665	-54.9	2510	-30.5	Bleeding ectopic pregnancy
254	December 1 1937	F	39	162.2	60.8	3,610	9.48	33.0	55	15	2210	-2.4	1170	-27.9	3480	-13.0	Bleeding fibroid
270	June 6 1937	F	48	155.0	36.8	3,300	9.94	32.4	40	15	2915	+19.2	1405	-23.8	5160	-16.3	Bleeding fibroid
285	February 11 1937	F	48	161.3	69.1	5,040	9.94	32.4	110	12	3070	+27.1	1405	-11.1	4320	-9.4	Bleeding fibroid
317A	October 8 1937	F	55	163.8	57.6	2,170	3.17	12.8	100	12	3070	+43.7	580	-72.0	3520	-12.6	Gastro-intestinal hemorrhage
317B	February 3 1939	F	57	162.5	65.8	2,220	4.10	14.2	110	15	3470	+30.8	930	-64.0	4050	+0.6	Treated with iron
391	March 6 1939	F	57	162.5	65.8	3,110	5.96	22.9	110	19	3150	+25.0	710	-55.6	3710	-7.3	Gastric hemorrhage
387A	January 26 1939	M	63	186.4		1,900	4.43	19.1	45	15	3540	+10.3	1890	-27.3	4960	-15.9	Bleeding duodenal ulcer
387B	January 30 1939	M	63	186.4		3,400	7.20	34.2	28	2970	+48.9	+10.3	1890	-27.3	4960	-15.9	On iron therapy
390	January 14 1939	M	52	161.3	56.0	1,875	4.04	10.1	17	3360	+28.9	389	-80.5	3740	-19.6	Gastric hemorrhage	
400	February 20 1939	F	37	157.4		1,875	4.40	18.7	75	17	2515	+16.2	575	-60.2	5090	-14.4	Gastro-intestinal bleeding
406	March 21 1939	F	48	157.2	55.4	2,625	6.62	22.2	75	13	2260	-2.0	640	-58.2	2900	-24.5	Bleeding duodenal ulcer
408A	March 23 1939	M	54	165.1	93.6	4,275	11.70	35.3	105	19	3850	+37.8	2150	-2.5	6000	+20.3	Bleeding duodenal ulcer
408B	March 30 1939	M	47	165.1	55.0	4,620	12.30	38.0	80	21	3670	+31.3	2240	-34.6	4530	+18.5	Bleeding duodenal ulcer
409	March 31 1939	M	47	165.1	55.0	3,590	10.35	31.7	40	19	3095	+9.7	1435	-79.6	4530	-9.2	Bleeding duodenal ulcer
410	April 3 1939	M	47	174.0	72.4	2,360	7.49	21.7	70	20	2770	-3.3	770	-59.1	4150	-23.9	Epistaxis—fracture of nasal bone
412	April 10 1939	M	50	172.7	67.4	3,230	3.23	29.3	13	3320	+3.5	760	-68.8	4080	-30.1	Bleeding duodenal ulcer	
415A	May 2 1939	M	42	172.5		1,910	6.85	18.6	34	3580	+15.1	2320	-61.3	3180	-19.5	Hematemesis	
415B	June 22 1939	F	60	175.0	70.6	4,740	12.50	39.1	14	2570	+5.5	1120	-29.1	3620	-8.4	Bleeding duodenal ulcer	
416A	May 24 1939	F	60	161.3		1,720	5.50	19.2	34	2570	+5.5	1120	-61.3	3180	-19.5	Bleeding duodenal ulcer	
EM 2A	March 9 1938	M	47	165.0	53.2	2,680	4.48	21.0	40	91	2925	+6.1	1700	-22.7	4330	-13.4	Post gastric resection
EM 2B	April 6 1938	M	47	165.0	59.5	4,100	8.95	29.3	30	13	2630	+22.1	1430	-35.0	4850	-3.0	Recurrent bleeding
EM 2C	May 26 1938	M	47	165.0	59.8	3,700	8.80	29.3	75	14	3430	+11.8	2290	-19.7	4520	-24.6	Recovered
EM 2D	March 2 1939	F	33	158.5	48.6	3,890	10.50	35.1	150	11	1880	-19.7	1020	-24.4	2500	-15.4	Bleeding fibroids
EM 4A	January 3 1939	F	33	158.5	48.6	3,890	10.50	35.1	150	11	1880	-19.7	1020	-24.4	2500	-15.4	Post curettage
EM 4B	February 13 1939	F	32	162.5	59.9	4,190	8.25	30.9	160	16	2725	+13.5	1220	-27.5	3945	-1.4	Hemorrhage
EM 5A	February 13 1939	F	32	162.5	59.9	4,190	8.25	30.9	160	16	2725	+13.5	1220	-27.5	3945	-1.4	Recovered
EM 5B	March 17 1939	F	32	162.5	61.6	4,990	10.50	40.4	39	18	2490	+3.7	1680	-5.0	4180	+4.5	Recovered

in anemia, since frequently the subjective symptoms of both conditions are very similar

MATERIAL STUDIED

Thirty-two patients with anemia from the Medical and Surgical Wards of the Peter Bent Brigham Hospital and 9 from the Robert Dawson Evans Memorial, Boston, were studied. Of these, 8 males and 12 females had anemias due to *blood loss*, 2 males and 9 females had *hypochromic anemias of unknown cause*, 3 males and 3 females had so-called *macrocytic anemias*, and 2 males and 2 females had *hemolytic anemias* due to congenital hemolytic jaundice, malaria or sulfanilamide intoxication. In 7 males and 9 females repeated determinations were made during recovery. None of these cases were in an acute hemorrhagic stage, sufficient time having elapsed between the onset of hemorrhage and the determination of blood volume to permit physiological compensation by tissue fluid to take place. Adequate fluid administration was carried out in this interval. In this study we were not concerned with volume changes immediately after acute blood loss. No patient in the group studied succumbed to anemia and many whose course was not followed made complete recoveries on iron therapy, transfusion, or both.

Of the 11 cases of polycythemia vera studied, 5 males and 3 females were from the Medical Service of the Peter Bent Brigham Hospital and 1 male and 2 females from the Robert Dawson Evans Memorial. In 2 males and 1 female repeated determinations were made during the course of treatment.

METHODS

Plasma and total blood volumes and hematocrits were determined by methods previously described (22), venous pressures by a direct manometric method (23), circulation time by means of decholin (24), and hemoglobin content of venous blood by a modification of the method of Osgood and Haskins (25). Height was taken as the basis for the prediction of normal blood volume. Normal values for hematocrit were taken as 44 per cent and 40 per cent (26), for red blood cell counts as 5,480,000 and 4,920,000 cells per cu mm of blood, and for hemoglobin content of venous blood as 15.4 and 14.8 grams per 100 cc. of whole blood (27), for males and females respectively.

RESULTS

Blood volume

Absolute plasma, circulating red cell and total blood volume in the 16 cases of anemia followed during recovery are shown in Table I in relation to the red cell count. Plasma volume was above the range of average normal value in all except 3 cases. In those cases with higher than normal volumes there was a general tendency for plasma to decrease as the red cell count rose. In 2 of the cases with subnormal values plasma volume increased during recovery. Circulating red cell volume was below normal in all cases and increased as the red cell count rose. There was a definite tendency towards a direct relationship between the degree of lowering of red cell volume and anemia.

At the time of the initial determination only 5 cases had a total blood volume above normal. During recovery total blood volume fluctuated but there was a general tendency for the total blood volume to increase in the cases in which subnormal values were found and to decrease in those in which higher than normal values were found at the initial determination, and with 3 exceptions total blood volume was within the range of normality at a level of from 4.5 to 5.5 million red cells.

Considered from the point of view of predicted normal blood volume for the individual, there was some discrepancy in plasma, circulating red cell and total blood volume in relation to the red cell count as illustrated in Figure 1. While the average trend of plasma volume was to be above normal at a low red cell count, decreasing during recovery, the spread of cases above and below normal was considerable. This characteristic was not peculiar to any one of the etiologic groups.

Circulating red cell volume was below the predicted normal red cell volume for males and females in all but 1 case (Case EM-9A with macrocytic anemia) at the time of the initial determination. The trend toward a direct relationship between the deficit in red cell volume and level of anemia, indicated by the average line in Figure 1, was much more definite than in the case of plasma volume.

Total blood volume was subnormal in all but 6 instances at the time of the initial observation and

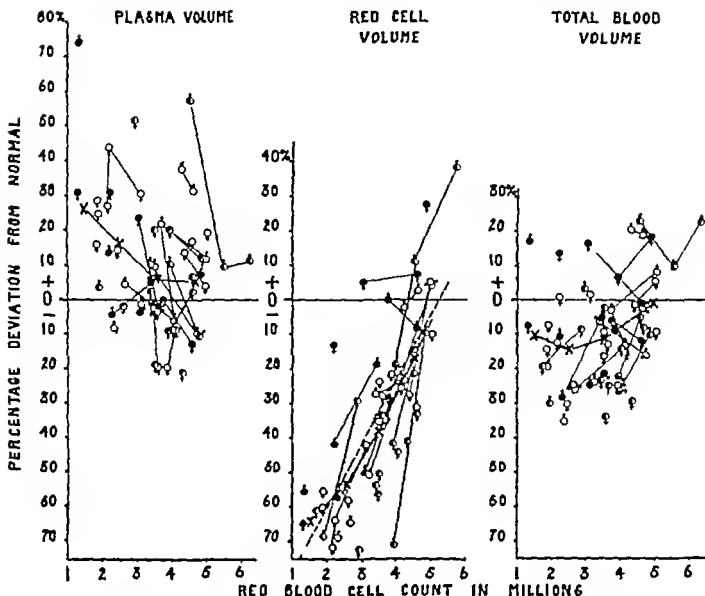


FIG. 1. CHANGES IN BLOOD VOLUME IN SECONDARY ANEMIA IN RELATION TO NORMAL

Symbols refer to etiologic groups as follows: anemia due to blood loss ○, hypochromic anemia, cause unknown ⊙, macrocytic anemia ●, and hemolytic anemia ⊗. While there are wide variations in volume, the average trend in secondary anemia, indicated by the heavy black lines, is for plasma volume to be above and total blood volume to be below normal in severe anemia, due to a greatly diminished red cell volume. As the red blood cell volume increases the relationship of changes in plasma and red cell volume is such that the increase in total red cell mass bears a direct relationship to the erythrocyte level.

the trend was to be low until the red cell count rose to approximately 4 million cells. However, the relationship between total blood volume and level of anemia was distinctly less evident than in the case of the circulating red cell volume.

The data obtained in the cases of polycythemia vera are summarized in Table II. In the untreated stage total blood volume was well above normal for the individual without exception as shown in Figure 2 and the increase was definitely in relation to the red cell count. Plasma volume was below normal in 7 and above normal in 4 cases when first observed; the average trend bearing no relationship to the red cell count. Circulating red cell volume was definitely increased in all cases, being greater than 150 per cent above normal in 6

of the 11 cases in the untreated stage. Case 383 with a red cell count of 14,065 million cells had a circulating red cell volume of 388 per cent above normal. The linear relationship between the degree of increase above normal in total circulating red cell volume and red cell count was equally as striking as in the cases of anemia.

Treatment consisted of repeated phlebotomies in 2 cases. No significant change in plasma volume resulted but large reductions in circulating red cell and hence in total blood volume, were effected and these followed closely to the general trend in relation to red cell count. In Case EM-14 the removal of a total of 8,000 cc. of blood by repeated phlebotomies during a period of about 11 weeks reduced the circulating red cell volume

TABLE 1—Continued

Case number	Date	Sex	Age	Height	Weight	Red cell count	Hemo-globin	Hemato-crit	Ve-nous pres-sure	Cir-cula-tion time	Plas-ma vol-ume	Per-centage devia-tion from normal	Red cell vol-ume	Per-centage devia-tion from normal	Total blood vol-ume	Per-centage devia-tion from normal	Remarks
			years	cm	kgm	mil-lions per mm ³	grams per 100 cc	per cent of cells	mm H ₂ O	sec-onds	cc	per cent	cc	per cent	cc	per cent	
11 CASES OF HYPOCHROMIC ANEMIA, CAUSE UNKNOWN																	
45A	June 21, 1935	F	32	163.8	36.8	3.520	4.14	22.5	75	11½	2380	— 1.5	790	— 50.8	3170	— 21.2	Chlorosis
45B	July 17, 1935	F	32	163.8	37.2	3.620	7.87	35.3	75	21	1950	— 19.2	1070	— 33.5	3020	— 24.9	Chlorosis
1118	December 2, 1935	F	36	160.0	48.0	4.650	9.20	28.9	65	12	2580	+ 9.1	1050	— 33.3	3630	— 7.9	Chlorosis
1137	January 14, 1936	F	25	171.5	67.5	3.520	11.5	29.0	115	17	2990	+ 20.1	1070	— 35.5	4060	— 2.2	Chlorosis
1171	April 12, 1936	F	28	152.4	38.6	3.420	5.80	23.0	40	9½	2220	+ 2.5	665	— 54.0	2885	— 23.3	Chlorosis
256A	December 4, 1936	M	60	174.3	55.4	3.950	10.20	17.5	70	23½	3340	+ 10.6	710	— 71.3	4050	— 26.4	Duodenal ulcer—malnutrition
256B	April 5, 1939	F	63	174.3	71.0	4.820	17.20	45.4	70	27	2700	— 10.6	2240	— 9.7	4940	— 10.2	Recovered
261	December 21, 1936	F	66	158.8	44.0	2.960	10.03	32.6	70	18	3560	+ 51.8	430	— 72.5	3990	+ 3.2	Chlorosis
402	February 27, 1939	F	30	174.0	62.4	4.020	10.03	32.6	70	18	2780	— 8.9	1340	— 44.3	4120	— 24.4	Duodenal ulcer
EM 6A	April 29, 1938	M	30	183.0	77.7	4.540	6.00	28.1	65	12	5120	+ 57.6	2000	— 21.8	7120	+ 22.8	Duodenal ulcer—post gastric resection
EM 6B	June 4, 1938	M	30	183.0	77.7	4.540	6.00	28.1	65	12	5120	+ 57.6	2000	— 21.8	7120	+ 22.8	Duodenal ulcer
EM 6C	January 1, 1939	M	30	183.0	77.7	4.540	6.00	28.1	65	12	5120	+ 57.6	2000	— 21.8	7120	+ 22.8	Duodenal ulcer
EM-7A	October 20, 1938	F	26	154.9	55.5	3.920	15.93	49.4	90	16½	3560	+ 9.6	2815	+ 10.4	6375	+ 9.9	Chlorosis
EM-7B	November 21, 1938	F	26	154.9	55.5	3.920	7.40	29.9	120	12	2045	— 9.1	870	— 42.0	2915	— 22.1	On iron therapy
EM 8	September 14, 1938	F	62	158.0	55.9	4.570	11.60	35.6	110	17½	2300	+ 2.2	1270	— 15.4	3570	— 4.8	Malnutrition
EM-11	May 7, 1939	F	18	154.7	36.1	3.560	5.75	26.3	110	15	1845	— 21.1	915	— 41.3	2760	— 29.2	Ulcerative colitis
6 CASES OF MACROCYTIC ANEMIA, CAUSE UNKNOWN																	
277	January 21, 1937	F	37	173.7	54.6	2.210	9.80	30.9	135	24½	3275	+ 31.0	1455	— 13.2	4730	+ 13.3	
278	January 28, 1937	F	68	166.4	67.2	1.310	5.24	15.1	135	24½	3200	+ 30.9	570	— 65.0	3770	— 7.5	
280	January 29, 1937	M	64	165.5	84.1	1.350	4.00	16.7	35	19½	4900	+ 74.2	980	— 55.7	5880	+ 17.0	
291	February 18, 1937	M	76	178.0	69.6	2.220	10.07	28.5	35	25	3600	+ 13.7	1440	— 42.0	5040	— 10.8	
EM-9A	February 1, 1939	F	62	159.0	58.6	3.050	12.00	36.1	45	13	2900	+ 23.9	1640	+ 5.1	4540	+ 16.4	?Plummer-Vinson syndrome
EM-9B	March 1, 1939	F	62	159.0	57.0	4.610	12.90	40.6	45	16	2290	— 2.1	1570	+ 0.6	3860	+ 1.0	
EM-10A	May 19, 1938	M	63	172.5	62.0	2.300	7.10	25.5	75	16	2920	— 4.3	1000	— 58.5	3920	— 28.3	Gastritis
EM-10B	May 19, 1938	M	63	172.5	62.0	2.300	7.10	25.5	75	16	2920	— 4.3	1000	— 58.5	3920	— 28.3	
EM-10C	June 26, 1938	M	63	172.5	63.6	3.440	9.56	36.5	75	15	3210	+ 5.2	1955	— 18.6	5165	— 5.2	
4 CASES OF HEMOLYTIC ANEMIA																	
172-A	April 18, 1937	F	26	155.0	48.6	4.600	11.70	41.2	90	14	1955	— 13.1	1375	— 8.3	3330	— 12.0	Congenital syphilis
172-B	May 1, 1937	F	26	155.0	48.6	4.600	11.70	41.2	90	14	1955	— 13.1	1375	— 8.3	3330	— 12.0	Post malarial anemia
172-C	May 1, 1937	F	26	155.0	48.6	4.600	11.70	41.2	90	14	1955	— 13.1	1375	— 8.3	3330	— 12.0	Tertiary syphilis—post malarial anemia
199	March 19, 1937	M	41	173.6	57.0	3.830	10.20	36.2	60	23	3280	+ 7.0	1700	— 29.6	4480	— 9.0	Congenital hemolytic anemia
282A	February 6, 1937	F	17	172.6	55.2	3.960	13.52	30.3	85	15	3100	+ 20.2	1350	— 18.9	4450	+ 6.5	2 years post splenectomy
282B	April 4, 1939	F	19	172.6	56.7	4.880	13.85	43.1	85	18	2800	+ 12.2	2120	+ 27.3	4920	+ 18.3	Sulfanilamide toxicity
417	May 29, 1939	M	15	172.6	46.6	3.090	9.08	29.8	90	15	2880	— 3.7	1220	— 50.3	4100	— 24.6	

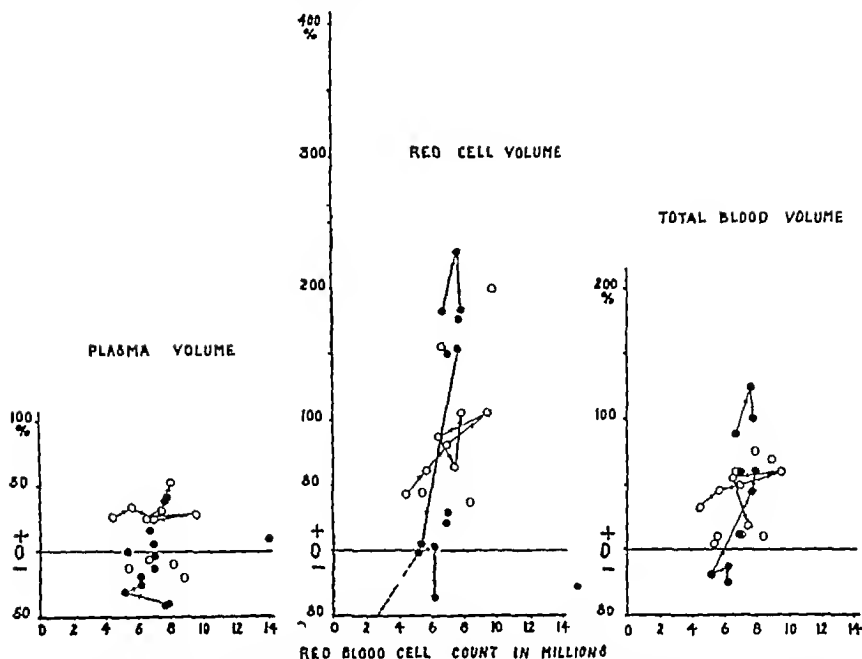


FIG. 2. BLOOD VOLUME IN POLYCYTHEMIA VERA

Black circles represent males and open circles females. Solid lines indicate the changes in individual cases. Total blood volume is greatly increased due to an increase in circulating red cell volume. The average trend of increase in red cell volume constitutes a continuation of the line representing a direct relationship between the level of total red cell mass and red blood cell count obtaining in secondary anemia.

from 183 per cent above to 35.6 per cent below normal and total blood volume from 59.2 per cent above to 26.3 per cent below normal

Circulation time

Circulation time tended to be fast in anemia and slow in polycythemia as indicated in Figure 3. In the former group in 9 instances the circulation time was slower than 20 seconds and in 20 faster than 15 seconds while in the latter group of 16 observations 4 were slower than 20 seconds and only 4 faster than 15 seconds. None of these patients showed any evidence of congestive heart failure or of metabolic disturbance. When both groups were considered as a whole there was a continuous slowing in circulation time accompanying an increase in erythrocyte level. In the group

of anemias no one etiologic group exhibited any particular deviation from the general trend

Venous pressure

In anemia venous pressure varied between 30 and 150 mm. of water averaging about 50, and in polycythemia vera between 30 and 115 mm. of water averaging about 40. There was no relationship between the venous pressure and total blood volume during the severe stages or during periods of clinical improvement in either anemia or polycythemia vera.

DISCUSSION

From these results it seems reasonable to conclude that secondary anemia is characterized by a low total blood volume, due primarily to a great

TABLE II
Findings in 11 cases of polycythemia vera

Case number	Date	Sex	Age years	Height cm	Weight kgm	Red cell count millions per mm ³	Hemo- globin grams per 100 cc	Hemo- crite per cent of cells	Ve- nous pres- sure mm Hg	Cir- cula- tion time sec- onds	Plas- ma vol- ume cc	Per- centage devia- tion from normal	Red cell volume cc	Per- centage devia- tion from normal	Total blood volume cc	Per- centage devia- tion from normal	Remarks
14	March 4, 1935	M	59	167.6	59.0	7,060	21.18	69.5		19	2,510	-13.5	5,700	+150.5	8,210	+58.6	
251	November 28, 1936	F	77	173.0	46.6	8,910	16.35	71.7			1,965	-20.6	4,965	+200.5	6,930	+68.1	
267	December 31, 1936	M	56	165.0	88.7	7,050	19.70	51.0	60	28	2,690	-3.4	2,810	+28.3	5,500	+10.5	
273	January 8, 1937	F	60	166.4	57.0	8,220	23.55	50.5			2,200	-10.0	2,250	+38.0	4,450	+9.2	
307A	May 14, 1937	M	58	174.3	60.6	6,780	16.65	65.1	40	19	3,620	+16.4	6,760	+185.0	10,380	+87.8	Gout
307B	July 8, 1938				62.5	7,690	16.06	65.4		23	4,280	+38.3	8,090	+228.5	12,370	+123.8	
307C	July 8, 1938										3,690	+19.2	7,110	+192.5	10,800	+77.3	Volume after phlebotomy of 1,000 cc
307D	July 11, 1938				64.1	7,780	15.15	59.9	30	18	4,410	+42.4	6,590	+178.5	11,000	+99.1	
307E	July 11, 1938										4,290	+38.6	6,240	+163.2	10,530	+96.1	Volume after phlebotomy of 1,000 cc
320	November 24, 1937	M	58	170.0	79.5	6,980	15.40	47.3	95	17	4,290	+5.1	2,800	+20.2	5,920	+11.7	
379	October 19, 1938	F	62	164.5	64.2	6,770	18.55	64.5	45	29	3,120	-6.2	4,150	+156.1	6,430	+58.8	
383	December 5, 1938	M	72	185.0	79.1	14,065	39.10	78.0			2,280	+25.9	1,970	+43.2	4,570	+32.9	
EM-12A	February 11, 1938	F	60	150.0	48.2	4,510	12.85	43.1	30	17	3,550	+8.2	12,550	+388.0	16,100	+175.2	
EM-12B	July 5, 1938				50.5	5,770	12.85	44.1	40		2,760	+33.7	2,210	+61.5	4,970	+44.5	
EM-12C	October 11, 1938				51.4	7,000	13.74	49.9	35		2,565	+24.2	2,555	+81.2	5,120	+48.8	After X-ray therapy over spleen
EM-12D	October 26, 1938				53.0	9,550	14.16	50.7	90		2,655	+28.8	2,845	+100.6	5,500	+59.8	
EM-12E	November 10, 1938				48.6	6,550	14.00	50.8	115	13	2,590	+25.4	2,635	+87.0	5,265	+53.1	After X-ray therapy over spleen
EM-12F	March 16, 1939				49.5	7,460	12.85	46.0	95	15	2,720	+31.7	2,315	+63.8	5,035	+17.3	
EM-12G	April 5, 1939				48.2	7,950	12.50	53.2	100	17	3,200	+55.0	2,820	+105.8	6,020	+75.0	
EM-13	May 23, 1938	F	55	157.5	61.0	5,540	16.00	52.6	75	14	1,830	-13.6	2,230	+43.8	4,240	+9.4	
EM-14A	June 9, 1938	M	58	172.8	52.7	7,770	20.90	78.8			1,760	-40.0	6,790	+183.0	8,620	+59.2	Volume after phlebotomy of 1,000 cc.
EM-14B	June 9, 1938										1,760	-42.3	6,080	+153.4	7,840	+43.8	After repeated phlebotomies to about 5,000 cc
EM-14C	June 30, 1938				51.4	5,200	14.15	53.4	40	16	2,065	-32.2	2,365	-1.5	4,430	+18.7	
EM-14D	July 14, 1938				54.1	6,200	11.82	52.1	55	13	2,270	-25.6	2,460	+2.5	4,730	+13.2	After repeated phlebotomies to about 2,000 cc
EM-14E	August 31, 1938				60.5	6,200	12.50	38.6	65	13	2,470	-19.0	1,545	-35.6	4,015	+26.3	

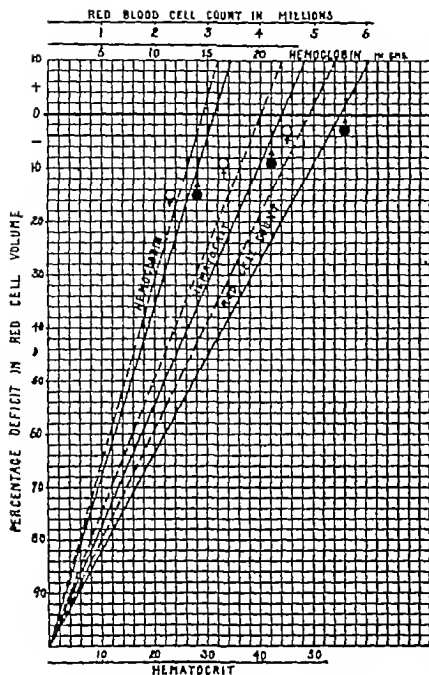


FIG. 4. NOMOGRAM FOR ESTIMATION OF DEFICIT IN CIRCULATING RED CELL VOLUME IN SECONDARY ANEMIA

red cell volume obtaining in a given case at any level of anemia. Furthermore some idea can be gained of the amount of whole blood that need be administered to restore the circulating red cell volume to normal. Chronic anemias of the type studied sometimes require transfusion as a life-saving procedure and no guide for estimating transfusion requirements is in common use. This requirement may be computed as follows. Normal circulating red cell volume may be derived from predicted normal total blood volume on the basis of either height or surface area, using the nomogram in a previous communication (26) and taking the hematocrit values as 44 and 40 for males and females respectively. The theoretical percentage of reduction from normal at any given level of red cell count hemoglobin or hematocrit may then be derived from the curves in Figure 4,

and the actual deficit in circulating red cell volume computed by multiplying the derived normal circulating red cell volume by this percentage. If the average hematocrit value of donor's blood be considered as 40 the total amount of whole blood required to restore the patient's circulating red cell volume to any desired level may be computed.

It should be emphasized that, while this computation is valid within the above stated limits for cases of chronic anemia, it may not be in cases of acute blood loss in the immediate post hemorrhagic period or while hemorrhage is continuing. Bennett, *et al* (11) followed the course of the blood volume by a dye method in 122 cases of severe gastro-intestinal bleeding and concluded that "assessments of the severity of anemia based on hemoglobin estimations are liable to grave error, particularly in the hours just following hemorrhage when dilution by plasma may by lowering the hemoglobin concentration, give a false idea of the true level of total red cell volume." They also stated that the "determination of blood volume is a real criterion of the severity of anemia" and that "the red cell portion of the blood volume is the all important factor." We feel that the dye method used by these authors is open to criticism (22 and 28). It is quite possible that the lack of correlation between hemoglobin and red cell volume noted may have been due to technical aberrations. We agree with these authors concerning the importance of the blood volume determination in assessing the severity of anemia and with the concept that the deficit in red cell mass is the one important fact from the clinical point of view in evaluating the severity of anemia.

It is worthy of comment that, as regards the state and course of blood volume in anemia with a few unimportant exceptions all anemias are essentially similar as shown in Figure 5. Regardless of etiology the circulating red cell volume exhibits the same characteristics in all anemias.

Our findings in polycythemia vera are in keeping with those previously reported. It is impressive to realize the truly enormous quantity of blood contained within the vascular bed of these patients, many of whom were cachectic and emaciated.

Haden (19) found that the red cell count did not accurately measure the total increase in red cells since it was "relatively too low." As shown in Figure 2 the relationship of plasma, circulating

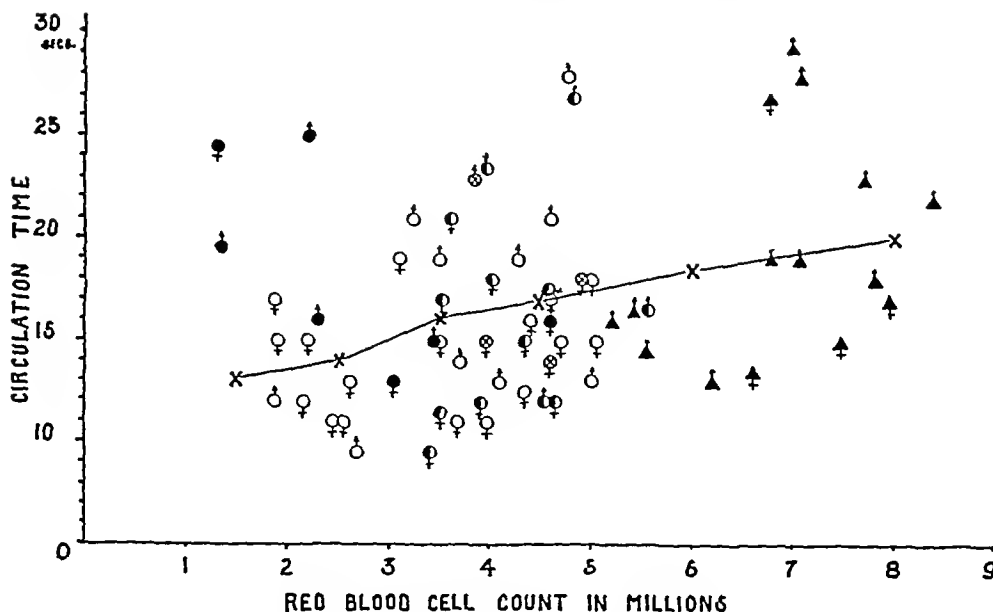


FIG 3 CIRCULATION TIME IN SECONDARY ANEMIA AND POLYCYTHEMIA VERA

Symbols refer to etiologic groups as follows anemia due to blood loss O, hypochromic anemia, cause unknown ◐, macrocytic anemia ●, hemolytic anemia ⊗, and polycythemia vera ▲ Circulation time becomes progressively slower as the erythrocyte level increases

reduction in the circulating red cell volume, despite an increased plasma volume. While in the individual case total blood volume may be within normal limits, the circulating red cell volume is below normal and the deficit in red cell mass is in direct relationship to the severity of the anemia as evidenced by the red cell count, hemoglobin determination or hematocrit level. During recovery there is an increase in circulating red cell volume and the relationship of changes in plasma and red cell volume is such that this increase tends to be directly proportional to changes in the red cell count, hemoglobin determination and hematocrit level.

This same relationship was observed in the cases of polycythemia vera. The theoretical slope of the direct relationship between the level of anemia and circulating red cell volume is shown in Figure 1 by the broken line. It is of some interest that the average of the deviation from predicted normal circulating red cell volume in the cases of polycythemia vera shown in Figure 2 constitutes a continuation of this line. Changes in circulating red cell volume effected by phlebotomy and occurring during relapses tended to follow this line closely.

This characteristic of the course of the circulating red cell volume in anemia offers a valid basis for the evaluation of the significance of the red cell count, hemoglobin determination and hematocrit as clinical guides to the severity of anemia in a given case. By reference to the curves for the theoretical direct relationship of circulating red cell volume to red cell count, hemoglobin or hematocrit shown in Figure 4, the theoretical deficit in red cell mass can be estimated. The agreement between this theoretical and the actually determined deficit in red cell mass should furnish a clue to the usefulness of the procedures in question. The best correlation obtains in the case of the hematocrit level in which in 61 observations 90 per cent of the cases fell within plus or minus 20 per cent and 69 per cent within plus or minus 15 per cent of the theoretical value. The correlation with the red cell count was less good and that with the hemoglobin determination was least good of the three procedures. For clinical purposes it would appear that the hematocrit gives a better index of the severity of anemia than does the red cell count or the hemoglobin determination.

With these facts in mind certain deductions can be made concerning the actual deficit in circulating

capacity of a unit mass of blood due to the red cell deficit and plasma dilution, the rate of flow is increased in relation to the lowered viscosity of the blood. During recovery the significant change is the increase in circulating red cell volume, and the relation of this increase to the course of plasma volume is such that total blood volume slowly rises from the subnormal level present in the severe stages to within normal limits for the individual when recovery is complete.

It is therefore evident that at critical levels of anemia, even though dehydration may be severe, the administration of fluids, especially intravenously, may by further dilution lower the oxygen capacity of the blood to dangerous and even fatal levels. This study also emphasizes the magnitude of reduction in circulating red cell volume in severe anemia and stresses the large amount of transfused blood necessary to raise red cell volume above critical levels. The great difference in normal total blood volume and hence red cell volume in males and females as well as those differences due to height and surface area must be considered in determining the amount of whole blood necessary for restoration of the circulating red cell volume to normal.

The technical procedures and time required for blood volume determination by the only satisfactory and reliable methods now available are such as to preclude them for routine clinical use, although the value of the determination in a critical case is obvious. Fortunately, there is a fairly good correlation between circulating red cell volume and hematocrit level in chronic anemia or in acute cases after hydration of the blood has occurred. The hematocrit is a better criterion of the degree of red cell volume deficit than either routine red cell counts or hemoglobin determinations.

In polycythemia vera the significant change is the tremendous increase in circulating red cell volume. It is apparent that therapeutic bleeding greatly diminishes the increased red cell mass and this fact justifies the use of this therapeutic measure in this disease. Marked clinical improvement resulted in the phlebotomized cases and symptomatic improvement was maintained as long as the total blood volume was kept down by repeated bleedings.

CONCLUSIONS

1 In chronic anemias, regardless of etiology, plasma volume is above and circulating red cell and total blood volume are below normal.

2 During recovery the relationship between the increase in circulating red cell and the decrease in plasma volume is such that total blood volume slowly increases, returning to within normal limits when recovery is complete.

3 For clinical purposes the hematocrit level is a better criterion of the degree of deficit in circulating red cell volume than the red cell count or hemoglobin determination.

4 Polycythemia vera is characterized by an increased total blood volume due entirely to a great increase in circulating red cell mass. The degree of this plethora is reflected in the erythrocyte level.

We wish to express our appreciation to Miss Evelyn Berstein for valuable technical assistance.

BIBLIOGRAPHY

1. Keith, N. M., Rowntree, L. G., and Geraghty, J. T., Method for determination of plasma and blood volume. *Arch. Int. Med.* 1915 16 547.
2. Hartwich, A., and May, G. Blutmengenbestimmungen mittels der Farbstoffmethode. *Technik untersuchung an normalen, polycythämien, anämien und chlorosen.* *Ztschr. f. d. ges. exp. Med.*, 1926, 51 497.
3. Griesbach, W., Eine klinisch brauchbare Methode der Blutmengenbestimmung. *Deutsche med. Wchnschr.*, 1921 47 1289.
4. Plesch, J., Untersuchungen über die Physiologie und Pathologie der Blutmenge. *Ztschr. f. klin. Med.*, 1922 93, 241.
5. Keith, N. M., Total circulating volume of blood and plasma in cases of chronic anemia and leukemia. *Am. J. Med. Sc.*, 1923 165 174.
6. Herzfeld, A., Über klinische Blutmengenbestimmung. *Munch. med. Wchnschr.*, 1922 69 1272.
7. Mendershausen, A., Blutmengenbestimmungen mit der Kongorotmethode. *Ztschr. f. klin. Med.*, 1923 97 468.
8. Smith, J. L., Discussion on the blood in disease. *Tr. Path. Soc., London*, 1900 51 311.
9. Robertson, O. H., and Bock, A. V., Blood volume in wounded soldiers: blood volume and related blood changes after hemorrhage. *J. Exper. Med.*, 1919 29 139.
10. Bock, A. V., Constancy of volume of blood plasma. *Arch. Int. Med.*, 1921 27 83.
11. Bennett, T. I., Dow, J., Lander, F. P. L., and Wright S., Severe hemorrhage from stomach and duodenum. *Lancet*, 1938 2 651.

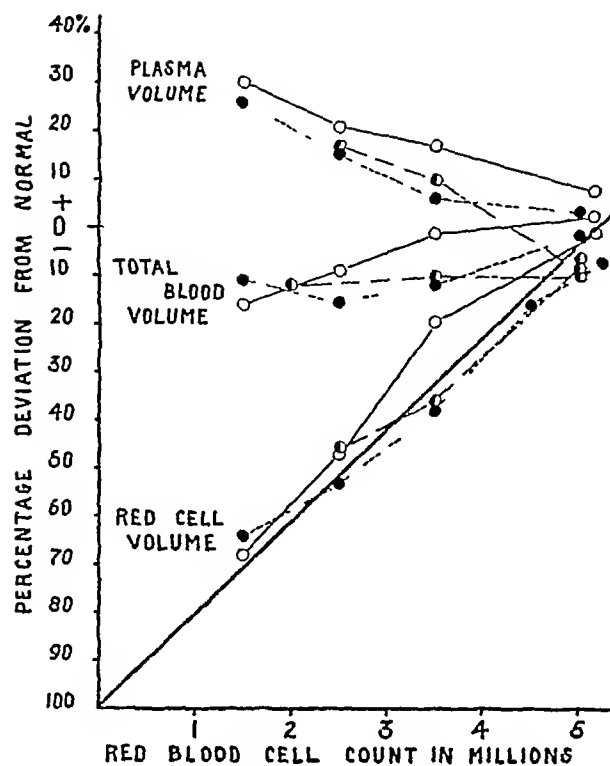


FIG 5 BLOOD VOLUME CHANGES IN ANEMIA

The symbols refer to the following classifications of anemia: pernicious anemia ○, anemia due to Bright's disease ●, and secondary anemia, all causes ●. There is a striking similarity in the deviation from normal in plasma, circulating red cell and total blood volume and the changes during recovery in all groups.

red cell and total blood volume in terms of percentage of normal to the red cell count reveals that the red cell count is a very fair indicator of the degree of elevation above normal of both circulating red cell and total blood volume. In the phlebotomized cases the changes in circulating red cell and total blood volume followed the general trend very closely during the periods of falling volume after bleeding and the periods of increasing volume during relapse.

None of the cases of polycythemia vera studied had congestive heart failure, elevated venous pressure or circulation times slower than 30 seconds. In comparison with a group of cases with congestive heart failure (29) in whom increases in both plasma and circulating red cell volume brought about an increase in total blood volume which was on an average 40 per cent above normal—only 6 cases out of 35 being more than 60 per cent above

normal—total blood volume in our cases of polycythemia vera was much higher, ranging from 92 to 175%, an average of 61% per cent above normal, without any appreciable variation from normal in plasma volume. The differentiating point seems to be that in congestive heart failure the plasma volume is greatly increased, whereas in polycythemia vera the plasma volume is essentially normal. In individual cases total blood volume may be as high in congestive heart failure as in polycythemia vera. For this reason the hematocrit becomes a useful diagnostic procedure, and at a hematocrit level of over 55 the diagnosis of polycythemia vera is justified in the absence of physical signs of congestive heart failure.

Venous pressures were within normal limits in cases of polycythemia vera and anemia. Variations therein bore no definite relationship to either changes in blood volume or clinical condition. Circulation times varied more widely than had been anticipated but in general confirmed the findings of other workers (30). The average trend bore a constant relationship to the red cell count suggesting that, in addition to other hemodynamic factors, the speed of blood flow is to a great extent determined by the viscosity of the blood (31). In cases followed during recovery the determination of circulation time added little, if anything, to the clinical evaluation of the case.

SUMMARY

A comparison of the blood volume findings of the 2 chronic blood dyscrasias herein studied reveals certain constant trends which are common to both. All chronic anemias, regardless of etiology or duration, are strikingly similar, a fact not to be inferred from the literature. In severe anemia, circulating red cell volume is greatly reduced and may fall as low as 60 per cent below normal before clinical signs of impending collapse become evident. In this stage the plasma volume is above the normal amount for the individual in a state of health and, whether this increase be considered as purposeful or not, it has the effect of maintaining the total amount of blood available for filling of the vascular bed and maintenance of an adequate relationship between vascular volume capacity and content. Coincident with the lowering of hemoglobin concentration, and hence oxygen

THE NON-SPECIFICITY OF SUSPENSIONS OF SODIUM XANTHINE IN PROTECTING THE LIVER AGAINST INJURY BY CHLOROFORM AND THE PROBABLE CAUSE OF ITS ACTION¹

By ISIDOR S. RAVDIN, HARRY M. VARS, AND SAMUEL GOLDSCHMIDT

(From the Harrison Department of Surgical Research and the Department of Physiology University of Pennsylvania Philadelphia)

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Sato (1a) has reported the extraction from the liver of a "detoxicating hormone," which he named *Yakriton*. This preparation is claimed to protect the body against a variety of induced pathological conditions, including the damaging effect of chloroform upon the liver (1b).

More recently Forbes and Neale (2a) and Forbes, Neale and Scherer (2b) have reported the preparation of a new fraction of an aqueous extract of hog's liver. When this extract was injected subcutaneously into albino rats previous to exposure to carbon tetrachloride or chloroform hepatic necrosis did not result. It also prevented the hepatic cirrhosis produced by repeated exposures to carbon tetrachloride (2b, 2f). A crystalline material identified as sodium xanthine by Neale (2d) and Neale and Winter (2e) was prepared from the extract by Forbes and Mc Connell (2c). Either the purified crystals or sodium xanthine, injected in equal quantities into rats conferred the same degree of protection against hepatic damage by carbon tetrachloride or chloroform (2d, 2e).

Barrett, MacLean and McHenry (3) have confirmed these findings in rats exposed to carbon tetrachloride. They injected either a suspension of purified 'antinecrotic' material furnished by Dr. Forbes or pure xanthine in equal dosages. Control injections of saline gave negative results. In agreement with Forbes and Neale and their co-workers they found both protection of the liver from damage and an accelerated regeneration of the injured cells.

Fitzhugh (4) has also reported protection by xanthine of the livers of rats exposed to carbon

tetrachloride. The outstanding effect of the xanthine was apparent 48 hours after the administration of the carbon tetrachloride, and consisted of more limited and smaller areas of necrosis than in the control rats. No evidence was found that xanthine stimulated the regeneration of the hepatic cells.

We have repeated and confirmed the experiments upon the protection offered to the liver by sodium xanthine against injury by chloroform and have extended our investigations with the view of testing its specificity and the mode of its action.

EXPERIMENTAL METHODS

Male and a few female albino rats 6 to 9 months of age, reared on Purina Chow in our own colony were used. In some of the experiments rats which had been deprived of food for 24 hours were used; such animals are very susceptible to hepatic injury by chloroform (5a). In the larger number of the experiments the rats employed had been transferred from the Purina Chow to a diet high in fat (our diet Number II) 9 to 18 days before use. The livers of rats upon this diet nearly always become necrotic after 1 hour of anesthetization with chloroform. All injections were made subcutaneously into the shoulder 22 to 24 hours preceding the anesthesia. The animals were uniformly anesthetized for 1 hour and sacrificed 24 hours later. The technique of administering the chloroform, the composition of diet Number II and the chemical and histological methods may be found in a previous publication (5a).

The histological data are reported as degeneration when it alone was present. Necrosis when found is reported as such even though degenerative changes were also observed.

EXPERIMENTAL

Suspensions of sodium xanthine. Sodium xanthine was injected in amounts of 50 mgm. in 1 ml. of water for each 100 grams of body weight of the rat. Since its solubility is slight this represented mostly suspended material. Twenty-three rats (Group Number 2) treated in this way and anesthetized with chloroform are to be

¹ Preliminary reports of the data in this paper were made before the Physiological Society of Philadelphia Am. J. Med. Sc. 1939 197 538, and the American Physiological Society Am. J. Physiol., 1939 126 646.

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- 12 Harrop, G A., and Heath, E H, Pulmonary gas diffusion in polycythemia vera. *J Clin. Invest.*, 1927, 4, 53
- 13 Brown, G E., and Giffin, H Z, Studies of vascular changes in cases of polycythemia vera *Am. J Med Sc.*, 1926, 171, 157
- 14 Lampe, W, Blutmengenbestimmungen bei Polycythemia Vera (Vaquez) *Deutsche med. Wchnschr.*, 1925, 51, 2025
- 15 Beltz, L., and Kaufmann, E, Symptomatologie und Therapie der Polyzythemie. *Klin. Wchnschr.*, 1924, 41, 1855
- 16 Parkes Weber, F, Polycythaemia, erythrocytosis and erythraemia *Quart. J Med.*, 1908, 2, 85
- 17 White, W H, Three cases of erythraemia (polycythaemia) *Lancet*, 1912, 1, 7
- 18 Rowntree, L G, Brown, G E., and Roth, G M, The Volume of the Blood and Plasma in Health and Disease. W B Saunders Co, Philadelphia, 1929
- 19 Haden, R L, The red cell mass in polycythemia in relation to diagnosis and treatment. *Am. J Med. Sc.*, 1938, 196, 493
- 20 Gibson, J G, 2d, Clinical studies of the blood volume. VI. Changes in blood volume in pernicious anemia in relation to the hematopoietic response to intramuscular liver therapy *J Clin. Invest.*, 1939, 18, 401
- 21 Harris, A W, and Gibson, J G, 2d, Clinical studies of blood volume. VII Changes in blood volume in Bright's disease with or without edema, renal insufficiency or congestive heart failure, and in hypertension *J Clin Invest.*, 1939, 18, 527
- 22 Gibson, J G, 2d, and Evans, W A., Jr, Clinical studies of blood volume. I Clinical application of method employing azo dye "Evans Blue" and the spectrophotometer *J Clin. Invest.*, 1937, 16, 301
- 23 Evans, Wm., Venous pressure. *New England J Med*, 1932, 207, 934
- 24 Winternitz, M., Deutsch, J, and Brüll, Z, Eine klinisch brauchbare Bestimmungsmethode der Blutumlaufzeit mittels Decholininjektion *Med. Klin.*, 1931, 27, 986
- 25 Osgood, E E., Haskins, H B, and Trotman, F E., Simplification of Osgood-Haskins hemoglobin method. *J Lab and Clin Med.*, 1931, 16, 482
- 26 Gibson, J G, 2d, and Evans, W A., Jr, Clinical studies of blood volume II The relation of plasma and total blood volume to venous pressure, blood velocity rate, physical measurements, age and sex in ninety normal humans *J Clin Invest.*, 1937, 16, 317
- 27 Murphy, W P, and Howard, I M., The iron content of crystals of hemoglobin prepared from human blood. (In preparation)
- 28 Gregersen, M I, Gibson, J G., 2d, and Stead, E. A., Plasma volume determination with dyes, errors in colorimetry, use of the blue dye T-1824 *Am. J Physiol (Proc.)*, 1935, 113, 54
- 29 Gibson, J G, 2d, and Evans, W A., Jr, Clinical studies of blood volume. III Changes in blood volume, venous pressure and blood velocity rate in chronic congestive heart failure. *J Clin. Invest.*, 1937, 16, 851
- 30 Blumgart, H L, Gargill, S L, and Gilligan, D R., Studies on velocity of blood flow XV Velocity of blood flow and other aspects of circulation in patients with "primary" and secondary anemia and in 2 patients with polycythemia vera *J Clin Invest.*, 1930, 9, 679
- 31 Cohen, M E, and Thompson, K. J, Studies on circulation in pregnancy I Velocity of blood flow and related aspects of circulation in normal pregnant women *J Clin Invest.*, 1936, 15, 607

mgm in each milliliter and each rat received 1 ml for each 100 grams of its weight. This dosage corresponded in its content of sodium to that contained in the sodium salts of xanthine.

The result of the injections (Group 8) of the sodium bicarbonate into 7 rats was entirely negative.

A 2.5 per cent solution of sodium chloride was injected into 3 animals (Group 9). Each rat received 1.5 mls (37.5 mgm). This strength of solution was chosen to control the factor of hypertonicity and the possible local irritation produced by some of the injected materials. The results, although admittedly inconclusive because of the small number of animals used, were not unlike those obtained with allantoin and caffeine in respect to the degree of protection afforded. The rather high concentration (1.04 per cent) and amount (0.119 grams per liver) of glycogen present in the livers 24 hours after the anesthesia are suggestive of a milder effect of the chloroform.

Barrett MacLean and McHenry (3) have reported negative results in experiments in which "saline" was injected as a control of the positive protective action of the "antinecrotic" material. The divergent results may be due to a difference in the concentration of the sodium chloride solution.

For reasons to be discussed later it was now decided to test the effect of injected materials having no remote relationship to xanthine or to the purines, but which produce different degrees of inflammation of the tissues with which they come into contact when injected under the skin.

Sodium ricinoleate. Fifty to 75 mgm of sodium ricinoleate per 100 grams of body weight, an arbitrary dosage was injected as a 10 per cent solution. In all cases 48 hours after the injection there resulted a nodular mass at the site of the injection, in a few instances an open abscess resulted. A group of 21 rats fed on Diet II, on which diet chloroform usually produced hepatic necrosis in 100 per cent of the animals (Group 1), exhibited no histological abnormalities of their livers 24 hours after anesthesia with chloroform (Group 10) when sodium ricinoleate was administered. The high hepatic glycogen concentration and amount are noteworthy.

Starved rats have been found to be more susceptible to hepatic injury at any given level of

lipid in their livers than fed animals (5a). In a control group of rats (Group 11), with 14.5 per cent of hepatic fatty acids, 80 per cent showed injury in their livers 70 per cent of which were necrotic. When sodium ricinoleate was injected into 9 similar animals under the same conditions, chloroform produced no histological changes in the liver of any animal of the series. Even in these animals which received no food before or after the anesthesia the concentration (0.75 per cent) and amount (0.047 grams per liver) were greater than in the controls (Group 11) which were not injected with sodium ricinoleate.

Colloidal carbon. Into another series of 15 starved animals 108 to 115 mgm of colloidal carbon which in contrast to sodium ricinoleate is chemically inert was injected for each 100 grams of weight of the rat. The carbon was present under the skin as a hard nodule 48 hours following the injection but in no case was there an open abscess. The incidence of liver damage following this treatment was 20 per cent (Group 13), as contrasted with 80 per cent in the control rats (Group 11), only 7 per cent of the livers of the animals which received the carbon were necrotic compared to 70 per cent in the controls. The hepatic glycogen in the control rats was but 0.54 per cent, with an average of 0.038 grams of glycogen in each liver, in the rats which received the carbon it was 1.39 per cent averaging 0.129 grams of glycogen per liver. This is an unprecedented high concentration of glycogen in the livers of unfed rats in our experience.

DISCUSSION

In the progress of the work detailed above it became apparent that the groups of rats which were protected to the greatest degree had received injections of suspended material: *i.e.* sodium xanthine and xanthine nitrate. In all of these animals definite nodules consisting of the suspended material and an inflammatory zone were present 48 hours after the injection. Histological examination of the tissue surrounding this area showed, in all cases examined, a marked degree of leukocytic infiltration. A milder degree of leukocytic infiltration was also found at the site of injection of solutions of allantoin and caffeine, due possibly in the case of sodium allantoin, to a deposit of crystals under the skin.

- 12 Harrop, G A., and Heath, E H, Pulmonary gas diffusion in polycythemia vera. *J Clin. Invest*, 1927, 4, 53
- 13 Brown, G E., and Giffin, H Z, Studies of vascular changes in cases of polycythemia vera *Am J Med Sc.*, 1926, 171, 157
- 14 Lampe, W, Blutnengenbestimmungen bei Polycythemia Vera (Vaquez) *Deutsche med. Wchnschr*, 1925, 51, 2025
- 15 Beltz, L, and Kaufmann, E, Symptomatologie und Therapie der Polyzythemie. *Klin Wchnschr*, 1924, 41, 1855
- 16 Parkes Weber, F, Polycythaemia, erythrocytosis and erythraemia. *Quart. J Med.*, 1908, 2, 85
- 17 White, W H, Three cases of erythraemia (polycythaemia) *Lancet*, 1912, 1, 7
- 18 Rowntree, L G, Brown, G E., and Roth, G M, The Volume of the Blood and Plasma in Health and Disease. W B Saunders Co, Philadelphia, 1929
- 19 Haden, R L, The red cell mass in polycythemia in relation to diagnosis and treatment. *Am J Med Sc.*, 1938, 196, 493
- 20 Gibson, J G, 2d, Clinical studies of the blood volume. VI Changes in blood volume in pernicious anemia in relation to the hematopoietic response to intramuscular liver therapy *J Clin. Invest*, 1939, 18, 401
- 21 Harris, A W, and Gibson, J G, 2d, Clinical studies of blood volume VII Changes in blood volume in Bright's disease with or without edema, renal insufficiency or congestive heart failure, and in hypertension *J Clin Invest.*, 1939, 18, 527
- 22 Gibson, J G, 2d, and Evans, W A, Jr, Clinical studies of blood volume. I Clinical application of method employing azo dye "Evans Blue" and the spectrophotometer *J Clin. Invest.*, 1937, 16, 301
- 23 Evans, Wm, Venous pressure. *New England J Med*, 1932, 207, 934
- 24 Winternitz, M, Deutsch, J, and Brüll, Z, Eine klinisch brauchbare Bestimmungsmethode der Blutumlaufszeit mittels Decholininjektion *Med Klin.*, 1931, 27, 986
- 25 Osgood, E. E., Haskins, H B, and Trotman, F E, Simplification of Osgood-Haskins hemoglobin method. *J Lab and Clin Med*, 1931, 16, 482
- 26 Gibson, J G, 2d, and Evans, W A, Jr, Clinical studies of blood volume II The relation of plasma and total blood volume to venous pressure, blood velocity rate, physical measurements, age and sex in ninety normal humans *J Clin Invest*, 1937, 16, 317
- 27 Murphy, W P, and Howard, I M, The iron content of crystals of hemoglobin prepared from human blood (In preparation)
- 28 Gregersen, M I, Gibson, J G, 2d, and Stead, E A, Plasma volume determination with dyes, errors in colorimetry, use of the blue dye T-1824 *Am J Physiol (Proc.)*, 1935, 113, 54
- 29 Gibson, J G, 2d, and Evans, W A, Jr, Clinical studies of blood volume III Changes in blood volume, venous pressure and blood velocity rate in chronic congestive heart failure *J Clin. Invest.*, 1937, 16, 851
- 30 Blumgart, H L, Gargill, S L, and Gilligan, D R, Studies on velocity of blood flow XV Velocity of blood flow and other aspects of circulation in patients with "primary" and secondary anemia and in 2 patients with polycythemia vera *J Clin Invest.*, 1930, 9, 679
- 31 Cohen, M E., and Thompson, K. J, Studies on circulation in pregnancy I Velocity of blood flow and related aspects of circulation in normal pregnant women. *J Clin Invest*, 1936, 15, 607

previous investigations to be discussed presently also pointed to the necessity of controlling this factor. Hence, the tests with sodium ricinoleate and the chemically inert colloidal carbon were made. The complete protection conferred upon the liver by the sodium ricinoleate and the superiority of the colloidal carbon in this respect over the sodium xanthine or other purines are convincing evidence that a mild local abscess or inflammatory process exerts a protective action in the situation under discussion.

The question arises whether the protection exerted by the sodium ricinoleate, the colloidal carbon and the suspensions of the xanthines is due to the same cause. Is it the result of the inflammatory process produced to a greater or lesser degree in all cases or does the inflammation or abscess liberate and make xanthine available to the body so that it becomes the common and specific factor?

The negative results following the injection of the filtrates from the saturated solutions of sodium xanthine and xanthine nitrate are suggestive but do not prove an ineffectiveness of xanthine solutions as compared to the suspensions. It may justly be argued that the dosage thus given is too small or that when so administered it is absorbed quickly as well as in small amounts compared to a possibly continuous dissolution and absorption of the suspended xanthine over the entire 48-hour period of the experiment.

The inference which one may draw from the publications of Forbes and Neale and their co-workers is that xanthine is the substance specifically responsible for the results observed by them and is the active constituent of their crude extract of the liver. However Neale and Winter (2c) claim to have obtained a degree of protection with a variety of purines in addition to xanthine—namely guanine hypoxanthine, and uric acid as well as with nucleic acid and its derivatives the nucleoside guanosine and a pyrimidine uracil. It must be concluded therefore that of the purines and nucleic acid derivatives xanthine is not a specific. It is noteworthy that all of these substances which gave a measure of protection to the liver were injected in suspensions because of their insolubility. We infer also that the material injected by Sato (1) was not entirely soluble. The degree of tissue inflammation and

damage is probably greater when suspended material is injected, with the consequent foreign body reaction than when a solution of the same substance is administered. The experiments with the colloidal carbon presented in this paper illustrate the effects produced by suspended inert material. On the other hand, an injected solution which is chemically an irritant may produce a profound reaction in the tissues to the point of abscess formation *i.e.*, solutions of sodium ricinoleate. It would appear that the protective value of the various unrelated materials studied by us and others is definitely a function of the degree to which they produced tissue inflammation and injury.

It is pertinent to the subject under discussion to examine the known effects of an inflammatory process or an abscess upon the body. Vaughan (6) in explanation of the excessive heat production responsible for the fever produced by the parenteral introduction of proteins into the body, suggested that it was due to the cleavage of the foreign protein, *in vivo* and also to a destructive reaction between these cleavage products and the proteins of the body, as evidenced by an increased nitrogen elimination. Unequivocal evidence that products of cell injury cause an increased protein catabolism in the body has been obtained in numerous experiments by Whipple and his collaborators. They found that the injection of toxic proteoses produced a great rise in urinary nitrogen and an increase in blood non protein nitrogen chiefly urea with slight amounts of amino peptide nitrogen (7). Cooke and Whipple (8) found that either sterile abscesses produced by turpentine injected under the skin or septic abscesses due to staphylococci also increased the output of urinary nitrogen and increased the blood non protein nitrogen. The explanation advanced to account for these effects is in general the same. The injected proteose produced a widespread cellular destruction in the body. The skin abscesses from turpentine or staphylococcus injection first caused a local injury of the tissues which in turn by the action of toxic split products absorbed from the abscess area resulted in the generalized injury and consequent increased protein catabolism. Cooke and Whipple (8) point out that the increased nitrogen elimination is too great to be accounted for by the local injury and tissue

compared with 18 control animals (Group Number 1) which did not receive sodium xanthine but which had been on the same diet for a comparable period of time, so that the average concentrations of hepatic glycogen and lipid were about the same before the anesthesia. The incidence of degeneration and necrosis of the liver due to the chloroform was reduced from 100 per cent in the control rats to 70 per cent in those previously injected with sodium xanthine. All of the livers of the control group exhibited areas of necrosis, while but 35 per cent of the group injected with xanthine showed necrosis. These results are in agreement with those of previous investigators in showing a protective action of an injected xanthine suspension.

Further evidence of a difference between these 2 groups of rats is found in the hepatic glycogen concentration following the anesthesia, it averaged 0.17 per cent or 0.019 grams per liver after the anesthesia in the control group, and 1.18 per cent or 0.117 grams in the rats which received xanthine previous to anesthetization. This is most probably a reflection of the lesser degree of injury by the chloroform in the latter animals (5b).

Solutions of sodium xanthine The filtrate of the saturated solution and suspended sodium xanthine used in the above experiments contained about 15 mgm of dissolved material at room temperature. One milliliter of this filtrate for each 100 grams of body weight was injected into Group Number 3, the rats were in all respects similar to those which received the suspension. Of the 13 rats so treated, all showed some degree of hepatic injury, 85 per cent of the livers were necrotic and the remainder showed degenerative changes. This result does not deviate significantly from the control figures (Group Number 1). The hepatic glycogen concentration and amount are not maintained after the anesthesia to the same degree as in the animals which received sodium xanthine.

Suspensions of xanthine nitrate and their filtrates Xanthine nitrate differs from the sodium xanthine in that, when suspended in water, it hydrolyzes to give an acid medium, the solubilities of the 2 salts do not differ greatly. When a suspension containing the same amount of xanthine used in Group 2 was injected into 6 rats (Group Number 4), 83 per cent showed hepatic

abnormalities, 50 per cent of which was necrosis and 33 per cent degeneration. This group, though small, indicates a protection, as compared to the control animals (Group 1), somewhat similar in degree to that afforded by suspensions of sodium xanthine. The difference in pH of the injected solutions appeared to have very little effect in altering the result. The concentration and amount of hepatic glycogen (1.37 per cent or 0.134 grams per liver) are indicative of a milder degree of hepatic injury in the treated rats.

When the suspended material was removed by filtration and the filtrate injected in the same volume, *i.e.*, 1 ml per 100 grams of body weight, all of the livers of the 7 rats so treated were necrotic (Group 5). As regards protection, the result is entirely negative. The hepatic glycogen concentration and amount (0.5 per cent or 0.052 grams per liver) are much lower than in the protected animal.

Sodium allantoin and caffeine In view of the negative findings with the saturated solutions of the relatively insoluble sodium xanthine and xanthine nitrate, tests were made with allantoin and caffeine, 2 soluble purines unrelated to xanthine. Fifty milligrams of allantoin in 2 mls of water solution, partly neutralized with sodium hydroxide, was injected for each 100 grams of the rat. Compared to the control rats (Group 1), the 9 rats which received allantoin (Group 6) showed the same total incidence of damage (100 per cent). The severity of the damage, however, was not so marked in the group injected with allantoin, 100 per cent of the livers of the controls were necrotic whereas but 56 per cent of the animals injected with allantoin showed necrosis.

In contrast to the findings of Neale and Winter (2e), the results here reported indicate a protective action of the allantoin.

Because of its pharmacological activity, caffeine was administered in much smaller amounts than the other purines, 17 mgm in 15 mls of water solution was injected for each 100 grams of the rat. The outcome (Group 7) is quite similar to that obtained with allantoin.

Sodium bicarbonate and sodium chloride solutions As a further control of the injections, tests for protection were made with solutions of sodium bicarbonate and of sodium chloride.

The sodium bicarbonate solution contained 13.2

previous investigations, to be discussed presently also pointed to the necessity of controlling this factor. Hence, the tests with sodium ricinoleate and the chemically inert colloidal carbon were made. The complete protection conferred upon the liver by the sodium ricinoleate and the superiority of the colloidal carbon in this respect over the sodium xanthine or other purines are convincing evidence that a mild local abscess or inflammatory process exerts a protective action in the situation under discussion.

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TABLE I
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Group number	Diet	Condition of rats	Control unanesthetized				Experimental anesthetized				Hepatic damage Degree and apportionment				Total damaged	Remarks
			Num ber of rats	Aver age glyco-gen liver*	Aver age glyco-gen per liver	Aver age fatty acids liver†	Num ber of rats	Aver age glyco-gen liver*	Aver age glyco-gen per liver	Aver age fatty acids liver†	Degenera tion		Necrosis			
				per cent	grams	per cent		per cent	grams	per cent	Num ber	per cent	Num ber	per cent	per cent	
1	II	Normal	14	1 97 (12)	0 212 (12)	26 1	18	0 17	0 019	23 2	0	0	18	100	100	
UNTREATED CONTROL RATS—DIET II																
2	II	Injected	9	1 04	0 103	21 3	23	1 18	0 117	25 6 (22)	8	35	8	35	70	
SODIUM XANTHINE SUSPENSION																
3	II	Injected	7	1 51 (5)	0 110 (5)	20 4	13	0 7	0 067	23 1	2	15	11	85	100	
SODIUM XANTHINE FILTRATE																
4	II	Injected					6	1 37	0 134	22 7	2	33	3	50	83	
XANTHINE NITRATE SUSPENSION																
5	II	Injected					7	0 5	0 052	27 1	0	0	7	100	100	
XANTHINE NITRATE FILTRATE																
6	II	Injected	1	1 62	0 162	27 3	9	0 81	0 90	24 1	4	44	5	56	100	
SODIUM ALLANTOIN																
7	II	Injected	1	2 04	0 232	26 7	9	0 64	0 065	26 9	3	33	6	37	100	
CAFFEINE																
8	II	Injected	2	1 85	0 150	20 5	7	0 47	0 050	22 6	0	0	7	100	100	
SODIUM BICARBONATE																
9	II	Injected					3	1 04	0 119	20 8	1	33	2	67	100	
SODIUM CHLORIDE																
10	II	Injected	1	1 28	0 140	19 9	21	2 08	0 223	25 2	0	0	0	0	0	
SODIUM RICINOLEATE RATS FED																
11	P C †	Normal	4	0 06	0 005	14 5										
	P C	Normal	6	0 49	0 029	14 3	10	0 54	0 038	16 9	1	10	7	70	80	24 hrs after food 48 hrs after food
UNTREATED CONTROL RATS NO FOOD DURING EXPERIMENT																
12	P C	Injected	2	0 05	0 003	18 3	9	0 75	0 049 (7)	18 7	0	0	0	0	0	
SODIUM RICINOLEATE NO FOOD DURING EXPERIMENT																
13	P C	Injected					15	1 39	0 129	19 7	2	13	1	7	20	
COLLOIDAL CARBON (HYDRO-KOLLAG) NO FOOD DURING EXPERIMENT																

* Glycogen is expressed as percentage of the wet weight of the liver

† Fatty acids are expressed as percentage of the dry weight of the liver

‡ Purina chow

Figures in parentheses indicate number of rats

It was this common factor of a local inflammatory reaction at the site of injection of suspensions of sodium xanthine, xanthine nitrate, and sodium allantoin, which led us to investigate the effect

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THE EFFECT OF QUANTITATIVE REDUCTION OF RENAL BLOOD FLOW UPON THE PREGNANT RABBIT

By L. V. DILL, C. E. ISENHOUR, AND J. F. CADDEN

(From the Department of Obstetrics and Gynecology Cornell University Medical College and the New York Hospital New York City)

(Received for publication July 27 1939)

In a recent paper (1) we described a syndrome which occurred in pregnant dogs and rabbits subjected to renal ischemia and which was characterized by hypertension, albuminuria, coma, convulsions and death. In most of the animals uremia and hematuria were also noted but these were not constant findings. Cardiac dilatation with acute congestion of all organs, and necroses in the liver, myocardium and kidneys were the usual findings at autopsy.

Bilateral constriction of the renal arteries rendered estimation of the actual renal flow so difficult that only very gross variations could be appreciated, and varying amounts of renal damage and nitrogen retention complicated the picture. A more precise quantitative method was needed in order to subject the animals to comparable amounts of renal ischemia.

Ryland (2) described the production of renal ischemia by reducing the blood flow through the aorta proximal to the points of origin of the renal

and acetic acid test and careful microscopic examinations were done on the urine after it had been centrifuged at low speed.

Measurements of the cell volume, red blood cells, and hemoglobin were done weekly. Studies of the cell volume were done by the Wintrobe (4) method; the red blood cells were counted in chambers, using pipettes approved by the Bureau of Standards; and hemoglobin estimations were made by the Sahli technic.

Chemical measurements of the blood were made at weekly intervals. Measurements of the non protein nitrogen were done by the method of Folin and Wu (5) of urea by the method of Van Slyke and Cullen (6) and of uric acid by the Folin (7) technic. Cholesterol was determined by the method of Leiboff (8) chlorides by the Whitehorn (9) method, and the carbon dioxide combining power by the method of Van Slyke (10).

Following the period of three weeks during which control studies were made the animals were subjected to laparotomy under aseptic technic. The aorta, which had been measured at laparotomy, was constricted above or below the points of origin of the renal arteries to varying degrees by means of a silver wire loop the inside diameter of which had been measured before application. The aorta of each animal with aortic con-

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				per cent	grams	per cent		per cent	grams	per cent	Num ber	per cent	Num ber	per cent	per cent	
1	II	Normal	14	1.97 (12)	0.212 (12)	26.1	18	0.17	0.019	23.2	0	0	18	100	100	
UNTREATED CONTROL RATS—DIET II																
2	II	Injected	9	1.04	0.103	21.3	23	1.18	0.117	25.6 (22)	8	35	8	35	70	
SODIUM XANTHINE SUSPENSION																
3	II	Injected	7	1.51 (5)	0.110 (5)	20.4	13	0.7	0.067	23.1	2	15	11	85	100	
SODIUM XANTHINE FILTRATE																
4	II	Injected					6	1.37	0.134	22.7	2	33	3	50	83	
XANTHINE NITRATE SUSPENSION																
5	II	Injected					7	0.5	0.052	27.1	0	0	7	100	100	
XANTHINE NITRATE FILTRATE																
6	II	Injected	1	1.62	0.162	27.3	9	0.81	0.90	24.1	4	44	5	56	100	
SODIUM ALLANTOIN																
7	II	Injected	1	2.04	0.232	26.7	9	0.64	0.065	26.9	3	33	6	37	100	
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8	II	Injected	2	1.85	0.150	20.5	7	0.47	0.050	22.6	0	0	7	100	100	
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10	II	Injected	1	1.28	0.140	19.9	21	2.08	0.223	25.2	0	0	0	0	0	
SODIUM RICINOLEATE RATS FED																
11	P C †	Normal	4	0.06	0.005	14.5	10	0.54	0.038	16.9	1	10	7	70	80	24 hrs after food
	P C	Normal	6	0.49	0.029	14.3										48 hrs after food
UNTREATED CONTROL RATS NO FOOD DURING EXPERIMENT																
12	P C	Injected	2	0.05	0.003	18.3	9	0.75	0.049 (7)	18.7	0	0	0	0	0	
SODIUM RICINOLEATE NO FOOD DURING EXPERIMENT																
13	P C	Injected					15	1.39	0.129	19.7	2	13	1	7	20	
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Bilateral constriction of the renal arteries rendered estimation of the actual renal flow so difficult that only very gross variations could be appreciated and varying amounts of renal damage and nitrogen retention complicated the picture. A more precise quantitative method was needed in order to subject the animals to comparable amounts of renal ischemia.

Ryland (2) described the production of renal ischemia by reducing the blood flow through the aorta proximal to the points of origin of the renal arteries. It seemed that the utilization of this method with the application of a clamp of known diameter to the previously measured aorta should give a fairly accurate estimation of the relative reduction of blood flow through the kidneys.

METHOD

Six pregnant and eight non pregnant female rabbits of 8 pounds or more in weight and of varying breeds were used as the experimental animals in which to constrict the aorta proximal to the points of origin of the renal vessels and four pregnant animals of the same type were used to control the effect of simple aortic constriction by placing the clamp distal to the points of origin of these arteries. These animals were obtained from a local dealer and were observed for three weeks before the experiment was begun.

For an additional period of three weeks blood pressure was recorded three times weekly by an indirect method (3).

Urine was collected in metabolism cages and examined weekly. Specific gravity was determined by a calibrated urinometer. Albumin was tested qualitatively by the heat

and acetic acid test, and careful microscopic examinations were done on the urine after it had been centrifuged at low speed.

Measurements of the cell volume, red blood cells, and hemoglobin were done weekly. Studies of the cell volume were done by the Wintrobe (4) method; the red blood cells were counted in chambers using pipettes approved by the Bureau of Standards; and hemoglobin estimations were made by the Sahli technic.

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The non pregnant control animals in which the aortae were constricted proximally and distally to the origin of the renal arteries were divided into four groups of two animals each. The cross-sectional areas of the aortae of these animals were constricted to approximately one-sixth, one-fourth, one-half and two-thirds of the original value.

The pregnant experimental animals were similarly into three groups of two animals. The cross sectional areas of the aortae, distal to the origins of the renal arteries were to one-fourth, one half and two-thirds of the measurement. The aortae of this group were constricted to one-sixth of the original cross section it was felt that pregnant animals renal injury would not survive long enough value. The aorta was constricted when the in the last half or in some instances the last period of gestation.

- (d) Whipple, G H, and Van Slyke, D D, Proteose intoxications and injury of body protein III Toxic protein catabolism and its influence upon non-protein nitrogen partition of blood. *J Exper Med*, 1918, 28, 213
- 8 Cooke, J V, and Whipple, G H, Proteose intoxications and injury of body protein IV The metabolism of dogs with sterile abscess, pancreatitis and pleuritis *J Exper Med*, 1918, 28, 223
Ibid Proteose intoxications and injury of body protein V Increase in non-protein nitrogen of the blood in acute inflammatory processes and acute intoxication *J Exper Med*, 1918, 28, 243
- 9 Davis, N C, Hall, C C, and Whipple, G H, The rapid construction of liver cell protein on a strict carbohydrate diet contrasted with fasting, mechanism of protein sparing action of carbohydrate *Arch Int. Med*, 1919, 23, 689
- Daft, F S, Robscheit-Robbins, F S, and Whipple, G H, Liver injury by chloroform, nitrogen metabolism, and conservation, liver function and hemoglobin production in anemia. *J Biol Chem*, 1936, 113, 391
- 10 Daft, F S, Robscheit-Robbins, F S, and Whipple, G H, New formed hemoglobin and protein catabolism, conservation of intermediates in anemic dog on a protein free diet. *J Biol Chem*, 1933, 103, 495
- 11 Daft, F S, Robscheit-Robbins, F S, and Whipple, G H, New formed hemoglobin and protein catabolism in anemic dog *J Biol Chem*, 1935, 108, 487
- 12 (a) Robscheit-Robbins, F S, and Whipple, G H, Infection and intoxication, their influence upon hemoglobin production in experimental anemia. *J Exper Med.*, 1936, 63, 767
(b) Daft, F S, Robscheit-Robbins, F S, and Whipple, G H, Abscess nitrogen metabolism in anemic and non-anemic dog, reserve stores of protein apparently involved *J Biol Chem.*, 1937, 121, 45
(c) Madden, S C, Winslow, P M, Howland, J W, and Whipple, G H, Blood plasma protein regeneration as influenced by infection, digestive disturbances, thyroid, and food proteins, deficiency state related to protein depletion *J Exper Med.*, 1937, 65, 431
- 13 Whipple, G H, Protein production and exchange in body including hemoglobin, plasma protein and cell protein. *Am J Med. Sc.*, 1938, 196, 609

during the operation. The urinary constituents of the non pregnant group were not altered, while both of the pregnant animals developed albuminuria and hematuria to a marked degree preceding delivery. A complete remission was noted in one animal following expulsion of the fetuses while in the other although the hematuria cleared up completely and the albumin was markedly reduced a moderate amount of albumin persisted until death occurred.

The autopsy findings on the pregnant animal of the moderately constricted group in which death occurred four days following delivery were healing abdominal wound, striate areas of necrosis in the cortices of both kidneys, gangrene of terminal ileum, fibrino-purulent peritonitis, lobular pneumonia of the right lower lobe, cloudy swelling of the liver, spleen and heart, cardiac dilatation and acute pulmonary edema. It was felt that recovery of the animal was imminent when infarction of the intestine occurred and that the terminal rise in blood nitrogen as well as the albuminuria and death were directly attributable to this accident.

The effect of constricting the aorta to two-thirds of its original cross-sectional area is shown in Table III. In both of the non pregnant and in one of the pregnant animals of this group there was at most a slight elevation of blood pressure. In the remaining pregnant animal however a marked rise occurred immediately following aortic constriction and persisted until one day before death. In neither of the gravid animals was there a significant change in non protein nitrogen or urea although in Animal A a slight decrease in uric acid occurred. Hemoglobin, red blood cell and cell volume values particularly in the non pregnant animals, showed a less marked decrease than occurred in the more severely constricted group. Albuminuria was noted only in the pregnant animals and delivery in Animal A caused the urine to become completely negative.

Autopsy findings on the pregnant animal of the minimally constricted group which died while resorbing the fetuses were cloudy swelling of the kidneys, focal necrosis of the liver and myocardium, cardiac dilatation, infarction of the spleen, infected pulmonary infarct of the right lower lobe with extension to the diaphragm, corpora lutea of the ovaries, and retained placental

tissue. We believe that death and pathologic findings in this animal are directly attributable to the syndrome produced by renal ischemia in the pregnant animal.

DISCUSSION

The differences in the clinical courses of these animals were quite apparent. At no time following operation did any of the non pregnant animals appear ill while without exception, the condition of the pregnant animals became steadily worse. With the advent of delivery the animal at once became much more lively, the fur immediately regained its glossy appearance, and within two days the animal was apparently normal. The degree of aortic constriction seemed to bear no direct relation to the appearance of the animal.

From these findings it seems logical to believe that the aorta of the non pregnant animal can safely be reduced to at least one fourth of its original cross sectional area without producing more than a persistent hypertension in the non pregnant female rabbit, yet a reduction to even two-thirds of the original cross sectional area may produce signs of toxemia and even death in the pregnant animal. That this toxic effect is due to reduction in renal flow and not to mere mechanical blockage is shown by the failure to produce significant changes in the course of pregnancy in animals in which aortic constriction is carried out below the points of origin of the renal arteries. Ryland (11) has shown that the blood pressure elevation which accompanies partial aortic obstruction does not occur when the obstruction is carried out below living kidney tissue and Brothner (12) has demonstrated that the removal of a large portion of the capillary bed as represented by the kidney causes no significant rise in pressure in animals in which the aorta is constricted at a point below the origins of the renal vessels.

We are unable to explain why the pregnant animal with severe restriction of renal blood flow fails to develop hypertension. Arterial spasm, which is usually considered the promulgator of hypertension is obviously not decreased in the pregnant animal but rather increased in a generalized fashion as evidenced by the widespread necroses in the heart, spleen, liver, and kidney of these animals whereas in the non pregnant animal

- (d) Whipple, G H., and Van Slyke, D D, Proteose intoxications and injury of body protein. III Toxic protein catabolism and its influence upon non-protein nitrogen partition of blood. *J Exper Med*, 1918, 28, 213
- 8 Cooke, J V, and Whipple, G H, Proteose intoxications and injury of body protein IV The metabolism of dogs with sterile abscess, pancreatitis and pleuritis *J Exper Med.*, 1918, 28, 223
Ibid. Proteose intoxications and injury of body protein V Increase in non-protein nitrogen of the blood in acute inflammatory processes and acute intoxication. *J Exper Med*, 1918, 28, 243
- 9 Davis, N C, Hall, C C, and Whipple, G H, The rapid construction of liver cell protein on a strict carbohydrate diet contrasted with fasting, mechanism of protein sparing action of carbohydrate. *Arch. Int. Med*, 1919, 23, 689
- Daft, F S, Robschert-Robbins, F S, and Whipple, G H, Liver injury by chloroform, nitrogen metabolism, and conservation, liver function and hemoglobin production in anemia. *J Biol. Chem*, 1936, 113, 391
- 10 Daft, F S, Robschert-Robbins, F S, and Whipple, G H, New formed hemoglobin and protein catabolism, conservation of intermediates in anemic dog on a protein free diet. *J Biol Chem*, 1933, 103, 495
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severe hypertension develops without producing any pathologic changes in these organs

That pregnancy produces this difference in reaction seems obvious from the observations that delivery or in some cases even the death of the fetuses, seems to effect an immediate recovery and that thereafter the animal responds as one of the non pregnant animals in which the aorta has been similarly constricted.

It is noticeable, but perhaps not remarkable, that abortion is much more frequent in these animals than in those animals previously reported (3) in which bilateral renal artery constriction and not aortic constriction was used to reduce the blood supply to the kidney. It is probable that the aortic constriction by further reducing the blood supply to the uterus as well as to the functioning luteal tissue, plays an important part in this tendency of the uterus to evacuate, especially since these organs as well as the fetuses are probably suffering from a rather marked anemia produced by the arterial spasm associated with this syndrome.

Hemoconcentration such as has been described in the human with toxemia of pregnancy (13, 14) is not noted in these animals, although it is probable that if it occurs it has been masked by operation and by the frequent taking of blood samples

CONCLUSIONS

A quantitative method for producing a relative reduction of blood flow through the kidneys is described.

The presence of pregnancy definitely increases the susceptibility of the rabbit to renal ischemia.

Minimal reduction of the blood flow to the kidney of the pregnant animal produces a clinical and pathologic syndrome which closely simulates "toxemia of pregnancy" in the human.

The authors wish to express appreciation to Dr. J. B. Pastore for valuable suggestions in the preparation of

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BIBLIOGRAPHY

1. Dill, L. V., and Erickson, C. C., Eclampsia like syndrome occurring in pregnant dogs and rabbits following renal artery constriction. *Proc. Soc. Exp. Biol. and Med.*, 1938, 39, 362.
2. Ryland, D. A., Pathogenesis of arterial hypertension in coarctation of aorta. *Proc. Soc. Exp. Biol. and Med.*, 1938, 38, 10.
3. Dill, L. V., Erickson, C. C., and Isenhour, C. E., Observations on the effect of renal ischemia on the rabbit in various stages of the reproductive cycle (In press).
4. Wintrobe, M. M., Macroscopic examination of blood. Discussion of its value and description of use of single instrument for determination of sedimentation rate, volume of packed red cells, leucocytes and platelets and of icterus index. *Am. J. M. Sc.*, 1933, 185, 58.
5. Folin, O., and Wu, H., A system of blood analysis. *J. Biol. Chem.*, 1919, 38, 81.
6. Van Slyke, D. D., and Cullen, G. E., A permanent preparation of urease and its use in the determination of urea. *J. Biol. Chem.*, 1914, 19, 211.
7. Folin, O., Improved method for determination of uric acid in blood. *J. Biol. Chem.*, 1930, 86, 179.
8. Leiboff, S. L., Improved apparatus for determination of cholesterol. *J. Lab. and Clin. Med.*, 1925, 10, 857.
9. Whitehorn, J. C., System of blood analysis. Supplement II. Simplified method for determination of chlorides in blood or plasma. *J. Biol. Chem.*, 1921, 45, 449.
10. Van Slyke, D. D., Studies of acidosis. II. Method for determination of carbon dioxide and carbonates in solution. *J. Biol. Chem.*, 1917, 30, 347.
11. Ryland, D. A., Renal factor in arterial hypertension with coarctation of aorta. *J. Clin. Invest.*, 1938, 17, 391.
12. Brothner, R. J., Hypertension from obstruction of aorta. *Proc. Soc. Exp. Biol. and Med.*, 1939, 40, 264.
13. Dreckmann, W. J., Comparative studies of the blood in the non-convulsive toxemias of pregnancy. *Am. J. Obs. and Gyn.*, 1933, 26, 543.
14. Pastore, J. B., The cell volume following delivery and its relation to blood loss and post partum infection. *Am. J. Obs. and Gyn.*, 1936, 32, 859.

PERIPHERAL VASCULAR ACTION OF ESTROGEN IN THE HUMAN MALE¹

By SAMUEL R. M. REYNOLDS AND FRANCIS I. FOSTER

(From the Department of Physiology Long Island College of Medicine Brooklyn New York)

(Received for publication August 3 1939)

The use of estrogen, in some women for the relief of the severe neurovascular disturbances of the menopause is an established therapeutic measure (1, 2, 3, 4, 5, 6) although its specificity may be doubted in certain instances (1, 7). In spite of its failure to act in all cases there is no doubt that in the majority of selected patients the relief is specifically attributable to the estrogen.

Injection of this hormone is followed by a reduction in the gonadotropic hormone content of the blood and urine (2, 6), and usually a subsidence of the vascular disturbances. We are uncertain as to whether the reduction in the amount of gonadotropin in the urine is directly related to the symptomatic relief, or only indirectly so through a more deep-seated response of the body to the hormone. Not all climacteric women having a high gonadotropin content in their urine suffer from neurovascular disturbances. Consequently the mechanism of the action of estrogen on the circulatory system remains to be defined. The present experiments were designed to obtain some objective measure that might be useful in this respect.

While it is generally recognized that estrogens are without demonstrable effect upon the mean level of arterial blood pressure (8, 9, 10, 11, 14, 15), evidence is accumulating to show that the effects of estrogen on the peripheral circulation are quite widespread throughout the body and are of a definite character. The following facts indicate this: the injection of estrogen causes swelling and changes in the water content of the sex-skin in certain primates (12); estrogen causes engorgement of the vessels in the nasal mucosa including that of women (13); the injection of estrogen in the human is followed by a fall in capillary pressure and dilatation of the nail bed

capillary vessels (14); estrogen causes a decrease of venous pressure in the hand (15); and, finally, estrogen causes a change in the water content of the skin of the rat within a few hours (16) which is qualitatively comparable to that occurring in the uterus and vagina (16, 17). This list is extended in the present work by observations on changes in skin temperature and in finger volume following the injection of estrogen. An increase in finger volume without venous occlusion is indicative of an augmented capacity of the small blood vessels in the fleshy parts of the finger, while changes in the temperature of the skin reflect alterations in the local rate of blood flow (18).

SUBJECTS PREPARATIONS AND METHODS

Since the observations were intended to be of a preliminary nature, adult human males were used in this study in order to establish a convenient objective criterion of the peripheral vascular action of estrogen. It was deemed unnecessary and perhaps unwise to use female subjects with normal menstrual cycles for a more complete study of the vascular actions of estrogen in menopausal women will logically follow this preliminary study. The subjects, twenty in all, were eighteen men in the third decade of life and two in the fourth decade, all believed to be in good health at the time and apparently normal in every respect. One subject (*J. d. P.*) however, had a positive chest plate three months after the experiment although it is not probable that incipient tuberculosis would affect, or be affected by, the estrogen.

Single observations on the effect of the estrogen were made on all subjects with the exception of three who received two injections of estrogen and one subject who received nine injections. In all but one of the positive cases noted in Table I, corn oil alone was used at least once. No individual received more than one injection of the hormone in any one week. The subjects either

¹ Supported by grants from the Committee on Endocrine Research of the National Research Council, the Committee on Scientific Research of the American Medical Association and in part by a grant from the Josiah Macy Jr. Foundation.

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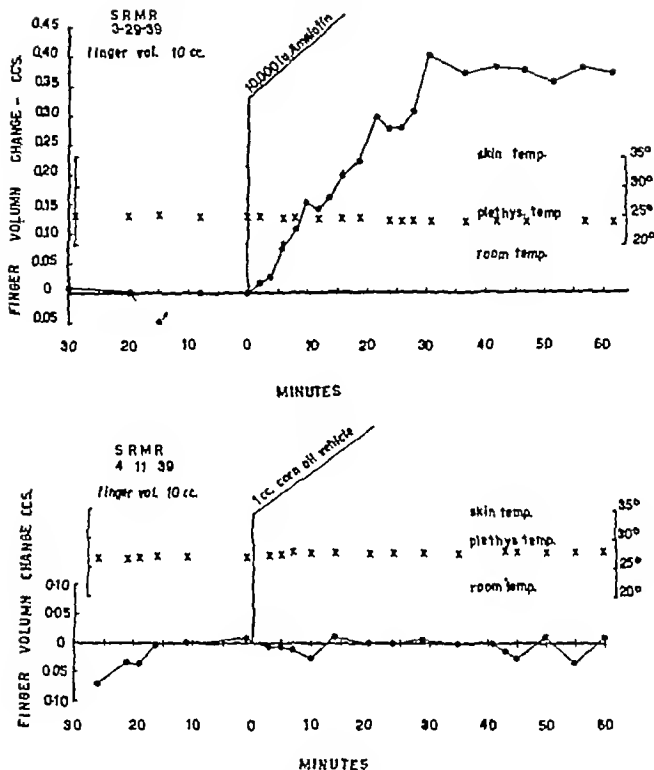


FIG. 1 RESPONSE OF INCREASED FINGER VOLUME TO THE INJECTION OF ESTROGEN IN CORN OIL (top) AND TO CORN OIL ALONE (bottom) IN A SUBJECT WITH A FINGER VOLUME OF 10 CC. IN THE PLETHYSMOGRAPH

features Within three to fifteen minutes the volume of the finger begins to increase not infrequently following a transient decrease (vasoconstriction) resulting from nervousness at the time of injection, the finger volume becomes steadily larger for at least half an hour, although more often the period of change lasts from forty five minutes to an hour, the finger stays large for a period in excess of the maximum time of observation (two hours)

The average gross increase was 0.67 cc. (average finger volume 14 cc.) although this varied considerably. In one subject (S. R. M. R., finger

volume, 10 cc.) the smallest change observed was about 0.15 cc. the maximum, about 0.55 cc. These observations were made between January and July although the magnitude of the response did not appear to vary with the season. In several subjects the response was as great as 0.9 to 1.0 cc. (finger-volumes of 12 to 14 cc) or a percentage change of 6.5 to 8.3. The average percentage increase in finger volume however was only 4.6. In order to appreciate the extent of this, it is necessary to note that the fleshy part of four embalmed fingers comprised 56 per cent of the total finger volume. It is to this part of

TABLE I

Summary of data from adult human males receiving estrogen intramuscularly

(See text for description)

Subject	Finger-volume	Increase	Latent period	Time to maximum	Postural adjustment skin temperature
	cc.	per cent	min utes	min utes	10 minutes standing
G.W.D.	12	3.25	6	60	
R.H.	12	3.83	6	36	
M.M.	18	3.00	2	15	
J.S.M.	14	7.50	3	20	
T.E.M.	14	6.65	2	37	-0.8° C
	20	13.50	2	40	
S.R.M.R.	10	3.80	1	30	-0.8° C
	10	2.50	10	25	
	10	2.70	4	33	
	10	5.00	10	50	
	10	2.10	18	38 (Stilboestrol used)	
	10	2.00	14	30 (Stilboestrol used)	
F.A.S.	10	6.40	7	40	-0.4° C
C.V.	10	8.30	3	48	-0.7° C
W.F.H.	15	4.80	3	37	-0.7° C
A.H.N.	12	3.30	7	50	
F.A.F.	21	0.80	2	20	
R.E.G.	11	(1.50)	6		(Leak in system, observation discontinued)
	11	(1.10)	2		(Leak in system, observation discontinued after 8 minutes)
E.B.D.	17	0			
B.W.	12	0			-0.4° C
C.L.S.	14	0			-1.0° C
J.F.S.	12	0			-0.6° C
J.d.P.	10	0			-0.0° C
	10	0			
F.W.H.	8	0			-0.1° C
H.C.M.	12	2.33	14	52	(Gave positive response to corn oil vehicle alone)
	12	2.10	6	27	
A.D.B.	20	1.70	4	30	(Gave response to corn oil vehicle alone)

knew nothing of what was being injected, other than that it was a harmless oily preparation, or they knew that estrogen or the inert oil vehicle was to be used. The exception to this was in early experiments on one of us (S R M R), at which time only the active hormone was available. After the first few observations the injections were made as unknowns.

The preparations used were Amniotin (Squibb), containing 10,000 (predominantly estrone) units per cubic centimeter, and the corn oil vehicle alone prepared in ampules exactly like those containing the estrogen. Stilboestrol (4,4'-dihydroxy α , β diethylstilbene, a synthetic compound having estrogenic properties), 10 mgm per cc, was used twice.²

In an experiment, the subject was seated comfortably, the arm supported snugly in a holder so that a finger was held without constriction in a volume-recorder of the type devised by Johnson (19). The recording droplet oscillated with each

pulse beat, with readings easily obtainable to 0.1 cc. Records were also kept of the temperature in the plethysmograph, the room temperature, the skin temperature measured on the nail of the finger next to the one in the apparatus. Temperature was recorded by means of a Beckman and Northrop potentiometer designed especially for measuring body temperatures and calibrated in degrees Centigrade and degrees Fahrenheit. The thermocouple was a twenty gauge iron-constantan junction, the system was sensitive to 0.1° C. Each experiment consisted of a period of observation recording lasting twenty to sixty minutes until conditions became constant (except two instances in which the subjects were nervous), in which case it was made intramuscularly into the triceps muscle of the free arm. Readings were made at five-minute intervals for a period of twenty to thirty minutes, after which readings were made at ten-minute intervals until from fifty minutes to an hour had elapsed.

RESULTS

The results noted in detail in Table I are summarized in Table II. Here it will be seen

TABLE II

General summary of data contained in Table I

Types of response to estrogen	Number of subjects	Number of responses to estrogen made	Response to corn oil	Average increase	Average latent period
				per cent	min utes
Positive response to estrogen	12	19	0	4.6	5.7
Negative response to estrogen	6	7	-	0	0
Positive response to estrogen and corn oil	2	3	+	2.0	8

two main types of response were obtained. In some subjects there was no discernible effect of estrogen upon finger-volume or skin temperature (six subjects) while in others (twelve subjects) the finger-volume increased appreciably, although there was no noticeable change in skin temperature. In general, the response had the folk-

² We are indebted to Dr J. A. Morrell for generous supplies of these preparations.

of blood in the finger. One must conclude therefore, that the increase in the volume of the finger after estrogen is not associated with a significant increase in the rate of blood flow, and that the heat dissipates rapidly as the finger volume change takes place slowly. It has been characteristically observed, however, that, in some subjects much more than in others, the initial phase of the rise in finger volume is associated with a transient feeling of warmth over the body and especially over the face, neck, chest and upper arms. Occasionally there is a feeling of clamminess as the subject perspires. At such times the finger volume may diminish owing to vasoconstriction resulting from the cool stimulus that necessarily follows loss of heat by rapid evaporation of sweat. This sensation should in no way be construed as a simulated menopausal flush since it is the normal response of the body to stimulation of warm receptors as the warm blood first moves into the skin. The mechanism is in all probability comparable to the paradoxical cold and warm sensations that can be elicited in normal persons under suitable circumstances (20).

Differing from either of the two types of response described above is a third which occurred in two subjects (H. M. and A. DiB.). In these cases an increase in finger volume was observed after injection of Amniotin although an equally large response was obtained after injection of the corn oil vehicle alone. Consequently these two subjects are not included in the group of individuals considered to have responded specifically to the estrogen.

DISCUSSION

Variations in response to estrogen. As noted in Table II approximately two-thirds of the subjects responded to injection of the estrogen. Six subjects showed no response whatever. The reason for this is not known. Unsuccessful attempts have been made to correlate the failure to respond with other conditions (e.g., blood pressure levels, skin coloring, constitutional type, vasomotor instability, tendency to perspire, sensitiveness to heat and cold, etc.). There is but one correlation that appears suggestive, namely the vasomotor adjustment that takes place in the skin of the lower leg during the first ten minutes of

standing from a reclining position (*cf.* 21). The data suggest that those subjects who fail to respond to estrogen show less marked vasoconstriction of the skin vessels (fall of skin temperature) upon standing than do those who respond to estrogen. One exception to this was noted, however, wherein the skin temperature diminished one degree Centigrade after eight minutes, yet the subject did not respond to the hormone (C. L. S.). This subject was by far the most muscular of the individuals used in these experiments, although the bearing of this fact on the problem at hand is not evident.

Whatever the reason for the failure of some individuals to give the peripheral vascular response to estrogen may be the fact remains that in a majority of the males studied in this series the response to estrogen was prompt in onset and definite in degree, while corn oil alone failed to give such a response. Whether or not the androgen level of a subject is a modifying factor, serving to potentiate or inhibit the vascular reaction to estrogen, is not known, although it is probable that this and other unappreciated endocrine factors exert a modifying action on the vascular response to estrogen.

Site of the vascular action of estrogen. A number of considerations point to the fact that the vasodilatation resulting from administration of estrogen involves the smallest blood vessels, namely the capillaries and venules of the skin. In the first place, if arteriolar dilatation were involved an increase in the rate of blood flow would occur and this would bring about a sustained increase in skin temperature. Such is not the case however. Secondly, Carlom observed (14) that dilatation of the nail bed capillaries after administration of estrogen occurs with a simultaneous decrease of capillary blood pressure. Systolic and diastolic blood pressures in the brachial artery are unaffected. This result could be accomplished in subjects with normal blood pressure only by vasodilatation beyond the arterioles. Lastly, direct observation of the action of estrogen administered intramuscularly to ovariectomized rabbits proved that vasodilatation in the ear of the rabbit involves the smallest blood vessels lying beyond the arterioles (22). These smallest vessels do not receive a sympathetic nerve

the finger that the vascular alterations are limited. Accordingly, the percentage increase is in reality about double the value stated here and in Tables I and II.

The skin temperature remained constant or fluctuated slightly, unless vasoconstriction occurred. The temperature of the plethysmograph likewise did not vary, despite the increased amount

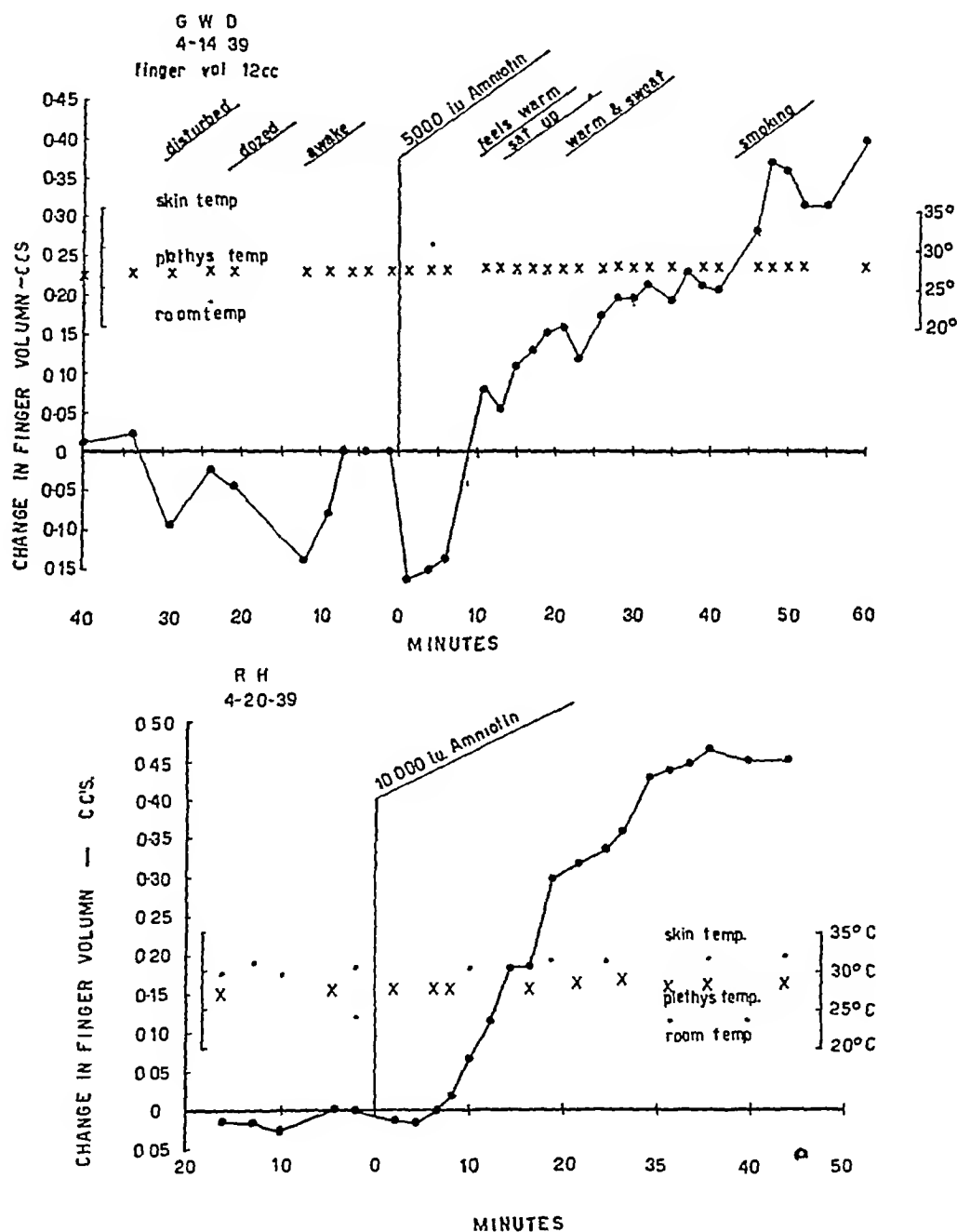


FIG. 2. RECORDS OF THE RESPONSE OF INCREASED FINGER-VOLUME TO ADMINISTRATION OF ESTROGEN IN TWO SUBJECTS

Subject G W D was nervous, as reflected in the variable base line during the control period and the fluctuations in skin temperature, indicating alterations in the rate of blood flow through the skin. Subject R. H. had a variable skin temperature at first, although this became more constant as the finger-volume increased to a plateau.

- 19 Johnson, C. A., Effect of amyl nitrite upon finger volume. *J Lab and Clin. Med.*, 1931-32, 17, 59
- 20 Bazett, H. C. Physiological responses to heat. *Physiol. Rev.*, 1927, 7, 531
- 21 Mayerson, H. S., and Toth, L. A., Influence of posture on skin and subcutaneous temperatures. *Amer J Physiol.*, 1939 125 474
- 22 Reynolds S. R. M., and Foster F. I., Peripheral vascular action of oestrin. *Amer J Physiol. (Proc.)* 1939 126, 606
- 23 Grant, R. T. Further observations on vessels and nerve of rabbits ear with special reference to effects of denervation. *Clin. Sc.*, 1935-36 2 1
- 24 Pompen A. W. M. De Invloed van Menformon op der Baarmoeder Thesis Amsterdam, 1933

supply (23), so it is necessary to conclude tentatively that the vasodilating action of estrogen in susceptible individuals is mediated by another, possibly direct, mechanism. It would appear to be significant in this respect that the dilating effect of estrogen on the vessels of the rabbit's ear may be inhibited by prior injection of atropine (22). This observation is in accord with the effect of atropine on the intense hyperemia which estrogen brings about in the uterus (14, 24). It may be, therefore, that the peripheral vascular action of estrogen upon certain parts of the systemic circulation resembles qualitatively, though in miniature, the recognized effects of estrogen upon the blood vessels of the uterus. Further work is necessary to render this conclusion certain, while its relation to the well-established effect of estrogen upon the neurovascular disturbances of the menopause remains to be demonstrated by direct observation upon women who require such treatment.

SUMMARY

1 The effect of intramuscular injection of estrogen upon the volume and skin temperature of the finger was measured in a group of twenty adult human male subjects.

2 Approximately two-thirds of the subjects showed an effect involving an increase in finger-volume, commencing a few minutes after injection and continuing from thirty to sixty minutes. A plateau level is attained which is sustained for the period of observation (maximum time up to two hours). The average percentage increase in finger-volume was 4.6. No change in skin temperature was noted in such cases.

3 Injection of the corn oil vehicle alone (as an unknown) had no such effect on finger-volume in these subjects.

4 The character of the response, along with other established facts regarding the vascular effects of estrogen, indicates that it depends upon dilatation of the small vessels in the skin beyond the arterioles. There is no measurable increase in the rate of blood flow in the skin.

5 The failure of estrogen to bring about dilatation of the skin vessels in some subjects is unexplained.

BIBLIOGRAPHY

- 1 Fluhmann, C. F., *Menstrual Disorders*. Saunders, Philadelphia, 1939.
- 2 Frank, R. T., Goldberger, M. A., and Salmon, U. J., Menopause symptoms, hormonal status, and treatment. *New York State J. Med.*, 1936, 36, 1363.
- 3 Albright, F., Studies on ovarian dysfunction. III. The menopause. *Endocrinology*, 1936, 20, 24.
- 4 Mazer, C., and Israel, S. L., Symptoms and treatment of menopause. *M. Clin. North America*, 1935, 19, 205.
- 5 Novak, E., Menopause and its management. *J. A. M. A.*, 1938, 110, 619.
- 6 Salmon, U. J., and Frank, R. T., Effect of emmenin on gonadotropic hormone excretion in castrates and spontaneous menopause. *Endocrinology*, 1937, 21, 476.
- 7 Pratt, J. P., and Thomas, W. L., Endocrine treatment of menopausal phenomena. *J. A. M. A.*, 1937, 109, 1875.
- 8 Parkes, A. S., *The Internal Secretions of the Ovary*. Longman's, Green and Co., New York and London, 1929.
- 9 Laqueur, E., Hart, P. C., and de Jongh, S. E., Ueber weibliches Sexualhormone (menformon) das Hormon des Östrischen Zyklus. IV. Einfluss auf den Stoffwechsel. Widerstandvermögen gegen physikalische und andere Eingriffe. *Deutsche med. Wchnsch.*, 1926, 52, 1331.
- 10 Kunde, M. M., D'Amour, F. E., Gustavson, R. G., and Carlson, A. J., Effect of estrin administration on reproductive and blood vascular systems: thyroid, thymus, hypophysis, adrenals, kidneys, liver and spleen. *Am. J. Physiol.*, 1931, 96, 677.
- 12 Aykroyd, O. E., and Zuckerman, S., Factors in sexual-skin oedema. *J. Physiol.*, 1938, 94, 13.
- 13 Mortimer, H., Wright, R. P., Bachman, C., and Collip, J. B., Effect of administration of oestrogenic hormones on nasal mucosa of monkey (*Macaca mulatta*). *Proc. Soc. Exper. Biol. and Med.*, 1936, 34, 535.
- 14 Carloni, E., L'azione degli estratti ovarici sull'atteggiamento dei capillari e sulla loro pressione, nelle varie fasi della rivoluzione funzionale utero-ovarica. *Arch. di ostet. e ginec.*, 1930, 17, 327.
- 15 Valle, G., Studio sulla pressione venosa in rapporto ad alcune condizioni normali patologiche e sperimentali della donna non gravida. *Ann. di ostet. e ginec.*, 1934, 56, 1011.
- 16 Zuckerman, S., Changes in the water-content of organs and tissues as a result of stimulation by oestradiol. *Nature*, 1939, 143, 521.
- 17 Astwood, E. B., Six-hour assay for quantitative determination of estrogen. *Endocrinology*, 1938, 23, 25.
- 18 Lewis, T., *Blood Vessels of the Human Skin and their Responses*. Shaw, London, 1927.

STUDIES ON DESTRUCTION OF RED BLOOD CELLS II CHRONIC
HEMOLYTIC ANEMIA WITH PAROXYSMAL NOCTURNAL
HEMOGLOBINURIA CERTAIN IMMUNOLOGICAL
ASPECTS OF THE HEMOLYTIC MECHANISM
WITH SPECIAL REFERENCE TO
SERUM COMPLEMENT¹

By THOMAS HALE HAM AND JOHN H. DINGLE²

(From the Thorndike Memorial Laboratory Second and Fourth Medical Services (Harvard)
Boston City Hospital the Department of Medicine and the Department of Bacteriology
and Immunology Harvard Medical School Boston)

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In previous publications (1, 2) certain prominent features of the mechanism of hemolysis *in vitro* have been described for patients with the syndrome of chronic hemolytic anemia with paroxysmal nocturnal hemoglobinuria Marchia fava Micheli disease (3, 4, 5). The erythrocytes from the 5 patients studied (2) were hemolyzed by all fresh human serums of compatible blood groups the degree of hemolysis being increased by acidification. Patients' serums however, produced no hemolysis of normal erythrocytes. The inhibitory effect on the hemolysis of heat and certain salts known to inhibit serum complement pointed to an association of the phenomena of lysis with complement. Consequently the question was raised was this hemolysis the result of an immunological reaction requiring complement? Augmentation of hemolytic activity by increased acidity did not rule out this possibility, since certain known antigen antibody reactions may be enhanced by acidification (6, 7). Nor did the loss of activity occurring with a small amount of dilution (1:4 or 1:5) militate against the hypothesis, for the system could be likened to one in which a small amount of antibody was present, thus requiring a large amount of complement for hemolysis to occur (8). This investigation was, therefore, undertaken to determine whether or not the ordinary components of an immune system—antibody complement and antigen—could be demonstrated.

Considering the hypothesis that a hemolytic antibody was concerned in the reaction there

were at least two possibilities: first that the antibody was present in serums of both patients and normal subjects, and second that the patient's erythrocytes were "sensitized" *in vivo* by a hemolytic antibody and required complement for hemolysis as suggested by Jordan (6).

For the sake of simplicity the data are presented in following order: (1) examination of serum for the presence of hemolytic antibody, (2) relation of complement to the mechanism of hemolysis, (3) antigenic properties of patient's and normal red blood cells, (4) examination of patient's red blood cells for hemolytic antibody, (5) comparison with human isohemolysins, (6) susceptibility of patient's erythrocytes to hemolysis in immunological systems and (7) susceptibility to hemolysis in non immunological systems. The case numbers correspond with those employed in the previous publication in which case reports were given (2).

GENERAL METHODS

Hemolysis of patients' red blood cells suspended in fresh serum *in vitro* did not necessarily occur at the natural pH of serum (1:2) but was always observed when the acidity of serum was increased by addition of certain acids. For this reason the hemolysis test was performed with acidified serum unless otherwise noted. In these data the term "hemolytic activity" refers to the degree of hemolysis of patients' erythrocytes in such a test. The complement concentration of serum is expressed in units per cubic centimeter.

In the hemolysis test with acidified serum described in detail previously (2) packed washed red blood cells from 0.5 cc. of a 5 per cent suspension were resuspended in plasma (heparin) or serum acidified by the addition of a 5 per cent by volume of 0.85 normal lactic acid or $\frac{1}{3}$ normal hydrochloric acid and incubated for 1 or 2 hours at 37° C. Under these circumstances

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for the presence of a hemolytic antibody which was any more readily absorbed than was serum complement either from the patient's serum or from normal serum. There was no demonstrable difference in this respect between patients' serums and normal serums. However, as with sheep cells when by repeated absorptions the complement concentration of either serum was materially reduced, its hemolytic activity for the patient's cells was correspondingly reduced. In these experiments the red blood cells from patients and from controls exhibited no apparent difference in absorptive activity. Normal cells and patients cells were not sensitized nor hypersensitized respectively, by treatment in the patient's serum (Table I). Contrary to the report of Dacie, Israels and Wilkinson (13), there was no evidence that previous chilling at 0° C. of mixtures of patient's cells and serum increased the subsequent hemolysis at 37° C. The methods employed in making these observations are described immediately below.

In further examinations for the presence of antibodies it should be emphasized that no cold agglutinins or isoagglutinins were ever observed at temperatures of from 0° C. to 37° C. for the cells and serum of patients with paroxysmal nocturnal hemoglobinuria or for normal cells of compatible blood groups suspended in their serums. Isohemolysins for the red cells of blood group II-A were encountered in the serum of Case I whose blood was of group IV-O.

EXPERIMENTAL

Absorptions with intact red blood cells were performed with samples of 3 cc. of serum from Case 3 and from a control. Each was absorbed at the natural pH of the serum by 1.5 cc. of washed packed erythrocytes both from Case 3 and from the control at 0° C. for 6 and for 15 hours. The serum from each of these 4 combinations was then acidified and tested for hemolytic activity. The treated serums showed the same lytic activity as unabsorbed serums for the erythrocytes of Case 3. The procedure was then repeated as above except that the serums were adjusted to a pH of 6.6 by the addition of 5 per cent of $\frac{1}{2}$ normal HCl and the two absorptions were performed for 3 hours each; the results were the same.

In 2 observations the serum from Case 1 was absorbed repeatedly at 37° C. with samples of 1.0 cc. of packed red blood cells from Case 1 and from a control, respectively employing 2.5 cc. samples of serum at its

natural pH. In the first observation with cells from Case 1 5 successive absorptions for 30 minutes each produced moderate hemolysis during the procedure. The hemolytic activity of the acidified serums for cells from Case 1 was before absorption, 13 per cent; after absorption with patient's cells, 7 per cent; after absorption with normal cells, 11 per cent. In the second observation, 5 absorptions of 15 minutes each were employed. The hemolytic activity of the acidified serums from Case 1 for homologous cells was before absorption, 11 per cent; after absorption with patient's cells, 11 per cent; after absorption with normal cells, 7 per cent. These variations were not considered to be significant.

Absorptions were made with stroma prepared by the method of Tharmhauser and Setz (14) from the erythrocytes of Case 3 and of a normal control. Samples of serum from Case 3 were acidified to a pH of 6.6 by the addition of lactic acid and absorbed for 90 minutes at 37° C. with 5 per cent by volume of washed packed stroma from both patient and control, respectively. These treated serums showed a slight but equal diminution of hemolytic activity for the cells of Case 3 and an equal decrease in complement titer from 48 to 12 units per cubic centimeter.

Exposure of packed red blood cells from patients to fresh homologous serum and to normal serum was carried out as follows: In one observation 0.25 cc. of cells from Case 3 were treated repeatedly at 25° C. for 10 minutes with samples of 1.0 cc. of fresh serum from Case 3 at its natural pH. The hemolysis of these cells when suspended in fresh samples of acidified serum was untreated cells, 11 per cent; cells treated once with fresh serum, 10 per cent; cells treated 6 times with fresh serum, 8 per cent. There was no evidence, therefore, of hypersensitization of patient's erythrocytes. In another ex-

TABLE I
Patient's erythrocytes not hypersensitized by own serum *

Serum mixture†		Hemolysis of red blood cells from Case 3 after treatment with serum or saline			
Active serum	Serum heated at 56° C. for 5 minutes	Heated serum from Case 3	Heated serum from normal subject	Saline	
per cent	per cent	per cent	per cent	per cent	per cent
100		9.0	10.0		12.0
80	20	4.0	4.0		6.0
50	50	0.3	0.1		1.5
40	60	0	0		1.0
30	70	0	0		0
Saline	0	0	0		0

* Washed packed erythrocytes from Case 3 were made to a 5 per cent suspension in serum (heated 56° C. for 5 minutes) from Case 3 from a normal subject and to saline. Mixtures were incubated 1 hour at 37° C. chilled 15 hours; the supernatant removed and hemolysis of treated cells tested with mixtures of active and inactive serum from Case 3.

† $\frac{1}{3}$ Normal hydrochloric acid 5 per volume, saline not acidified.

erythrocytes were never significantly hemolyzed. Venous blood defibrinated with beads was the usual source of serum and cells. As anticoagulant, heparin in a 15 per cent solution in distilled water was employed in a concentration of 130 mgm per 100 cc. of blood. This concentration does not inhibit complement. The degree of hemolysis was estimated by inspection or by determination of the hemoglobin concentration of the supernatant serum, employing a modification of the benzidine method of Bing and Baker (9) described previously. The action of normal isohemolysins was excluded by employing, as controls, blood samples of the same or of compatible blood groups.

The complement concentration was determined by incubation for 1 hour at 37° C of the mixture consisting of 0.5 cc. of a 2 per cent suspension of washed sheep cells, 2 units (0.5 cc. of a 1:2250 dilution) of anti-sheep cell rabbit serum, and 0.5 cc. of serum in graded dilutions. The highest dilution of complement giving complete hemolysis was considered as the endpoint. For the sake of numerical expression and comparison of potency, three times this dilution was considered as the number of units of complement per cubic centimeter, which must be regarded as a rough approximation due to the error in a titration of this type (10).

1 Examination of serum for hemolytic antibody

Fresh human serum either from the patients or from normal subjects was required for hemolysis of the patients' red blood cells. It was essential, therefore, to determine whether these serums contained a lytic substance which could be classified as a specific or non-specific antibody and which could be distinguished from the normal complement or alexin of fresh serum. The relationship of complement to the hemolytic mechanism is discussed in the next section. In the experiments reported below a series of observations was made comparing the hemolytic activity of samples of serum from Cases 1 and 3, normal infants, normal adults, and from patients whose serums contained abnormally elevated concentrations of hemolytic heterophile antibody. Finally, the serums were tested by certain absorption procedures known to remove antibodies and by methods designed to show the transfer of an antibody from serum to red blood cells (sensitization). In these procedures, however, no evidence was obtained for the presence of a hemolytic antibody in the serum of patients and of normal subjects.

a Infants' serums Since the serums of infants between the ages of 8 and 20 months are known to be relatively free from natural anti-

bodies (10, 11, 12), the hemolytic activity of 7 such serums was tested on the red cells from Case 3 and on cells from a normal control of the same blood group (I-AB). None of the infants were of type I-AB but none of the serums showed isohemolysins for the normal type I-AB erythrocytes. However, each acidified infant's serum produced significant hemolysis of the erythrocytes of Case 3 but not of normal erythrocytes. The degree of hemolysis of patient's cells was 10 per cent or greater for 3 serums, approximately 5 per cent for 2, and 3 per cent for 2. Complement titers varied between 24 and 48 units.

b Heterophile antibody The hemolytic heterophile antibody for sheep cells was entirely removed from the fresh serums of Case 3 and of normal adult controls by absorptions at 0° C with packed washed sheep cells. Such serums when subsequently acidified maintained their hemolytic activity for the erythrocytes of Case 3 if the serum complement had not been reduced materially. When both complement and heterophile antibody were removed by the sheep erythrocytes the serums were no longer hemolytic.

Four serums with heterophile antibody demonstrable by agglutination of sheep cells at dilutions of from 1:64 to 1:256 were obtained from patients convalescent from *infectious mononucleosis* and tested for their effect on erythrocytes from Case 3 and from a normal subject. None showed isohemolysins for group I-AB cells. The fresh acidified serum from one of the 4 patients produced no hemolysis of normal cells, however, and no more hemolysis of cells from Case 3 than was observed with a normal serum. The serums of all 4 patients produced no hemolysis of cells from Case 3 when 20 per cent by volume of whole guinea-pig serum was added as complement to the above serums which had been heated for 30 minutes at 56° C and then acidified. In this regard these serums were similar to normal serums as discussed in the next section.

c Absorption tests for hemolytic antibody In the following observations the serum from patients and normal controls was studied for the presence of hemolytic antibody by various absorption procedures. Either red blood cells or their stroma from Cases 1 and 3 or from appropriate normal subjects were utilized as potential absorbing agents. No evidence was obtained

EXPERIMENTAL

Absorption by sensitized or unsensitized influenza bacilli of serum from Case 3 and a normal subject decreased or removed complement, and correspondingly affected the hemolytic power of the treated serum. *Hemophilus influenzae* was grown on 12 agar plates and a heavy suspension made in 40 cc. of saline. To sensitize the organisms, equal volumes of suspension and anti-influenza rabbit serum diluted 1:40 were mixed and incubated 30 minutes at 37° C. The immune rabbit serum was prepared as described by Dingle, Fothergill and Chandler (10). For absorption of human serum, measured quantities of suspension were packed by centrifugation, the supernatant fluid discarded, and the organisms resuspended in serum. After incubation for 1 hour at 37° C. the serum was removed and acidified.

Standing at room temperature is well known to decrease the concentration of serum complement. Accordingly 2 normal serums and the serum of Case 3 were allowed to stand under sterile conditions at 25° C. for 5 days. By this means a reduction of complement from 48 units per cc. of serum to a trace was obtained. The hemolytic activity was absent from 2 and greatly reduced in the third serum. The addition of 20 per cent by volume of fresh undiluted guinea pig serum to the stored serums made no significant change in hemolytic activity but partially restored the complement concentrations to 24 units per cubic centimeter.

Repeated filtration through clean Berkefeld candles has been demonstrated by Strong and Culbertson (17) to remove all the known components of complement from fresh human serum. The thermolabile elements were the first to be removed and were not demonstrable in serum after from 3 to 6 filtrations. The heat-stable third and fourth components could still be detected after 9 filtrations.

In the present experiments after one Berkefeld filtration of the 4 serums tested, there was no measurable decrease in complement concentration, but the hemolytic activity was materially reduced in 2 instances and eliminated in 2 instances. The addition of 20 per cent by volume of fresh undiluted guinea pig serum restored the hemolytic activity completely in 1 instance, partially in 2 instances and failed to restore it in 1 instance.

After 5 passages through Seitz filters there was no detectable complement or hemolytic activity. The hemolytic activity however was not restored by the addition of 20 per cent by volume of fresh undiluted guinea pig serum.

c Inhibition of complement As reported in previous communications (1, 2), certain acids and salts (sodium citrate, citric acid, potassium oxalate and potassium cyanide) completely inhibited the lysis of the patients' red cells in the usual hemolysis test with acidified serum or plasma. Partial

inhibition was observed with oxalic and phosphoric acids and with heparin in concentrations greater than 130 mgm per 100 cc. of blood. Jordan (6) reported similar results in this disease with the above anticoagulants as well as with fluoride, hirudin and liqoid (Roche). Other investigators (18, 19, 20) have shown that the acids and salts referred to above inhibit complement action. There was a definite *parallelism*, therefore, between the inhibitory action of these agents both on complement and on hemolytic activity.

d Removal or inactivation of fractions or components of complement Complement can be separated by the proper procedures into globulin and albumin fractions and third and fourth components (21, 22, 23). The globulin fraction or midpiece and the albumin fraction or endpiece both contain thermolabile elements (7). These two fractions, however, contain the essential components of complement since their combination restores the full activity of complement. The third component can be removed by yeast or zymum (17, 22) or can be inactivated by cobra venom (24, 25). The fourth component can be inactivated by ammonium hydroxide or ammonium salts (23). The third and fourth components are relatively heat stable. Serums treated by such procedures have no apparent complement activity when used alone and recombination of these fractions restores complement action only when such mixtures contain qualitatively the above constituents, each in active condition. Accordingly serums from patients and controls were treated by procedures described below in order to separate, remove or inactivate these individual components of complement. Thereafter, the complement titer and hemolytic activity of the serums were determined before and after the addition of serum from the guinea pig and from certain other animals.

The globulin and albumin fractions after separation showed neither complement nor hemolytic activity when employed alone but both qualities were restored by recombination of the fractions.

EXPERIMENTAL

The albumin and globulin fractions of human serum were separated as described by Liefmann (21). The

* These suspensions were prepared through the courtesy of Dr. L. R. Seidman.

periment 5 per cent suspensions of washed red cells from Case 3 were made in heated serums (56° C. for 5 minutes) from Case 3 and from a normal subject, and in salt solution, respectively. The suspensions were incubated at 37° C for 1 hour and stored overnight at 5° C. After removal of the supernatant fluids the treated cells were resuspended in varying mixtures of heated and fresh acidified serum from Case 3 in the proportions shown in Table I. There was no evidence of increased sensitization of cells to hemolysis by previous exposure to the serum of Case 3.

2 Relation of complement to the mechanism of hemolysis

No evidence was obtained from the above observations that a hemolytic antibody was present in the serums examined. A serum factor was essential for hemolysis, however, and had many characteristics similar to those of serum complement as mentioned above. A prominent exception, however, to these resemblances between the serum factor and complement is the previously reported (1, 2, 13, 15) failure of fresh guinea-pig serum to restore the hemolytic activity of human serum heated at 56° C for 30 minutes. This problem is further investigated below, especially because Dingle, Fothergill and Chandler (10) have shown that certain immunological reactions require *human* complement and are not activated by animal serums. Accordingly, in order to compare further the behavior of the serum factor and of complement, the serums from 5 patients and from normal subjects were treated by procedures known to increase, decrease, or inhibit the action of complement as a whole or of one or more of the components of complement (7). Serums treated in this manner were then examined for their hemolytic activity for patients' red cells and for their concentration of complement.

a Increase of complement concentration The concentration of complement in human serum was increased by two procedures, first, the addition of guinea-pig serum, and second, the desiccation of human serum by lyophilization (16) and resolution of the dried material in one-half the original volume. The alkaline pH of lyophilized serum was adjusted by the addition of lactic acid and tested, as was the original serum, over a range of pH of from 6 to 8 (2).

In 13 of 15 observations the addition of 20 per

cent by volume of fresh undiluted guinea-pig serum to active human serum from *adults* produced a significant increase in complement titer, when compared to the original human serum, and a significant increase in hemolysis of the patient's erythrocytes but no hemolysis of normal erythrocytes. These results are in agreement with those of Dacie, Israels and Wilkinson (13). In 2 instances there was no increase in hemolysis of patient's cells. With *infant's serum* the results of the same procedure were not uniform. Although the complement titer was increased in all instances, a definite increase in hemolytic activity was observed in only 2 instances, a slight increase in 2, no change in 1, and hemolytic activity was entirely eliminated in 2 instances. There was no apparent explanation for the latter observation.

Lyophilized serum redissolved in its original volume contained the same apparent complement concentration as the original serum but its hemolytic activity was always decreased significantly. Lyophilized serum, which was redissolved in one-half its original volume and which contained twice the original complement concentration, produced either no hemolysis of patient's erythrocytes or only slight hemolysis. Thus lyophilization apparently decreased the lytic activity of human serum for patient's cells without demonstrable alteration of complement content.

b Decrease of complement concentration As already stated in section 1, when human serum was treated with sheep or human red cells or with the stroma of human red cells, the serum was rendered non-hemolytic for patient's erythrocytes in those instances in which complement was significantly reduced. In the following observations a direct study of the effect of decreasing the complement concentration was undertaken. The reactive components of complement were removed in part or completely from human serum by each of 3 procedures: absorption with sensitized or unsensitized influenza bacilli, standing at room temperature, or by filtration through Berkefeld or Sartz filters (17). The hemolytic activity of the treated serums was then tested on patient's erythrocytes and compared with the activity of an unmanipulated sample. In general there was a direct correlation between the complement concentration and hemolytic activity.

TABLE V

Comparison of human and guinea-pig complement *

Source of serum	Fresh human serum†	Human serum heated at 56° C. for 5 minutes	Fresh undiluted guinea pig serum‡	Hemolytic of red blood cells from Case 3	Hemolysis of normal red blood cells	Concentration of complement
	per cent of mixtures	per cent of mixtures	per cent of mixtures	per cent	per cent	units per cc.
Patient	100	100	20	+++	0	24
	50	30		trace	0	0
	20	80		0	0	24
		80	20	0	0	3
Control	100			30	trace	48
		100		0	0	0
	50	50		19	±	24
	30	70		10	0	12
	20	80		8	0	6
		80	20	0	0	48

* Effect of adding human serum and guinea pig serum to heat-inactivated human serum.

† Approximate hemolysis

+ 10 per cent

++ 20 per cent

+++ 30 per cent

‡ Lactic acid added (after heating) 5 per cent by volume, 20 millimolar concentration

EXPERIMENTAL

Human serum was heated for 7 minutes at various temperatures between 40 C. and 60 C. The results are shown in Table II. At 40 C. and 45 C. no significant loss of complement or hemolytic power occurred. At 50 C. complement was only moderately decreased in concentration but hemolytic activity was completely eliminated. At 55 C. and 60 C. both qualities were destroyed. As shown in Table III, serum heated at 50 C. for 7 minutes was not hemolytic when used alone and the lytic power was not restored by the addition of 20 per cent by volume of fresh human serum. Guinea pig serum however added to such heated serum in a concentration of 10 per cent by volume of undiluted fresh serum increased the complement titer and restored in part the hemolytic activity.

Human serum was heated at 56 C. for various periods of time from 1 to 30 minutes. The results are shown in Table IV. Serum so heated for 1 minute showed diminished complement concentration and no hemolytic power. The addition of guinea pig serum as employed above restored completely the complement titer and also restored in part the hemolytic power. After heating for from 5 to 30 minutes, however both complement and hemolytic activity were absent. Although the complement titer was restored by adding guinea-pig serum, the hemolytic activity was not restored to these mixtures.

Human serum and certain animal serums were further investigated as sources of the thermolabile components of complement. Mixtures of fresh human serum (20 to 50 per cent) and heat inactivated human serum showed

hemolytic activity which varied roughly as the complement titer as evident from the data in Table V (see also Table I).

Guinea pig serum was investigated for its effect in concentrations up to 50 per cent by volume of fresh undiluted serum added to heated human serum. Guinea pig serum in dilutions of from 1:5 to 1:40 was added to an equal volume of a 5 per cent suspension of patient's and of normal cells, respectively. For these experiments whole guinea pig serum was first absorbed with an equal volume of washed packed human erythrocytes for from 30 to 90 minutes at 0° C. in order to reduce the concentration of hemolysins of guinea pig serum for human cells. In these observations, such absorbed serum showed full complement activity but did not restore hemolytic activity to heated human serum, nor produce hemolysis when employed alone in the above dilutions. The same results were obtained in identical experiments employing unabsorbed serums from rabbit, dog, sheep and steer. When high concentrations of these unabsorbed animal serums were employed, hemolysis was observed in similar amounts for patient's cells and for normal cells of all 4 blood groups.

The third component of complement was removed from serum by zymun made from yeast. There was a direct correlation between the removal of hemolytic activity and of complement depending upon the volume of zymun and the time employed for absorption. Hemolytic activity always disappeared before complement was entirely removed. Combination of equal quantities of zymun treated and of heat-inactivated human serums showed only slight return of complement activity and no return of lytic power. It was impossible by the methods employed to restore a high concentration of complement in such mixtures. However whole untreated guinea pig serum did restore a high complement concentration to zymun inactivated human serum and in 2 of 4 observations restored in part the hemolytic activity to the zymun treated human serum.

EXPERIMENTAL

Serums from patients and controls were treated with measured amounts of zymun prepared from baker's yeast by the Strong and Culbertson (17) modification of the method of Whitehead, Gordon and Wormald (22). Zymun suspensions were centrifuged and the supernatant fluid was discarded. The packed zymun was then resuspended in the serums in concentrations of from 0.5 to 6 per cent by volume and the mixture incubated at 37 C. for from 15 to 30 minutes.

The fourth component of complement was inactivated by the use of ammonium hydroxide.

serum, diluted 1:10 with distilled water, was saturated with carbon dioxide at room temperature for 10 minutes and the precipitated globulin removed by centrifugation, washed 3 times with distilled water and dissolved in the same volume of saline as the original serum. The supernatant solution containing the albumin fraction was dried by lyophilization and redissolved in the same volume of distilled water as the original serum. When both fractions were recombined the precipitated globulin was dissolved in the albumin fraction, thus restoring the mixture to the same concentration of these constituents and of salts as existed in the original serum.

The thermolabile components of complement were inactivated in the following observations by exposure of human serum to varying temperatures. The hemolytic activity of serum disappeared before complement was measurably decreased in concentration or when only moderately decreased. Heated human serum, showing no lytic activity but definite complement activity, always showed partial restoration of lytic activity on the addition of fresh guinea-pig serum. When human complement was completely inactivated by heat, the hemolytic activity was eliminated. It was not restored either by small amounts of fresh human serum or by the fresh serums of certain animals including the guinea-pig. The data from representative observations are shown in Tables II, III, IV and V, and the procedures are described briefly below.

TABLE II

*Effect of heating serum for 7 minutes at 40–60° C**

Temperatures of serum heated for 7 minutes	Source of serum Patient (P) Control (C)	Hemolysis of red blood cells from Case 3	Hemolysis of normal red blood cells	Concentration of complement
degrees centigrade		per cent	per cent	units per cc
Unheated	P	16	0	48
	C	17	0	48
40	P	16	0	48
	C	15	0	48
45	P	13	0	48
	C	10	0	48
50	P	0	0	12
	C	0	0	12
55	P	0	0	0
	C	0	0	0
60	P	0	0	0
	C	0	0	0

* Hemolytic activity of human serum disappears before complement at 50° C

† After heating lactic acid added, 5 per cent by volume, 20 millimolar concentration

TABLE III

*Effect of adding fresh human serum and guinea pig serum to heated serum**

Source of serum Patient (P) Control (C)	Fresh human serum†	Human serum heated at 50° C for 7 minutes	Fresh undiluted guinea pig serum†	Hemolysis of red blood cells from Case 3	Hemolysis of normal red blood cells	Concentration of complement
	per cent of mixture	per cent of mixture	per cent of mixture	per cent	per cent	units per cc.
P	100			16	±	24
C	100			13	0	24
P		100		0	0	24
C		100		0	0	24
P	20	80		0	0	12
C	20	80		0	0	24
P		90	10	8	0	48
C		90	10	9	0	48

* Hemolytic activity was restored by guinea-pig serum and not by 20 per cent by volume of fresh human serum when added to human serum partially inactivated by heat

† Lactic acid added, 5 per cent by volume, 20 millimolar concentration

‡ Acid added after heating

TABLE IV

*Effect of adding guinea-pig serum to human serum heated at 56° C**

Source of serum Patient (P) Control (C)	Fresh human serum†	Length of time human serum heated at 56° C	Heated human serum†	Fresh undiluted guinea pig serum†	Hemolysis of red blood cells from Case 3	Hemolysis of normal red blood cells	Concentration of complement
	per cent of mixture	minutes	per cent of mixture	per cent of mixture	per cent	per cent	units per cc
P	100	0			17	0	12
	80	0		20	19	0	24
C	100	0			13	0	24
	80	0		20	20	0	24
P			100		0	0	6
		1	80	20	2	0	24
C			100		0	0	6
		1	80	20	10	0	24
P			100		0	0	0
		5	80	20	0	0	12
C			100		0	0	0
		5	80	20	0	0	24
P			100		0	0	0
		10†	80	20	0	0	24
C			100		0	0	0
		10†	80	20	0	0	24

* Hemolytic activity was restored by guinea-pig serum when human complement was only partially inactivated

† Serum heated for intervals of 15, 20 and 30 minutes gave results identical with those heated for 5 and 10 minutes

‡ Lactic acid added (after heating), 5 per cent by volume, 20 millimolar concentration

hydroxide to produce a serum pH of 9.0 to 9.1, the complement was similarly inactivated and was restored in part by the addition of an equal volume of serum inactivated by heat (56 C. for 5 minutes). The serum treated with sodium hydroxide had no hemolytic activity for patient's cells.

e Influence of the hemolytic reaction on complement concentration From the above observations it was evident that complement or a complement-like substance was closely related to the hemolytic activity of serum. It was important, therefore, to investigate whether complement was fixed or utilized during the hemolytic reaction. When samples of acidified serum from Case 3 or from a normal subject were treated repeatedly with packed washed red blood cells from either subject, hemolysis of patient's cells occurred in decreasing amounts with each exposure to fresh cells. There was as usual, no significant hemolysis of normal cells. After 4 such exposures, the complement concentrations of both the patient's and the normal serums were decreased to one-half the original quantity and the serums were materially diminished in hemolytic activity for patient's cells. No evidence was obtained, therefore, for specific complement fixation or utilization by the process of hemolysis of patient's cells, since normal human cells (without hemolyzing) removed complement at the same rate.

3 Antigenic properties of patient's and of normal red blood cells

The red blood cells of patients with paroxysmal nocturnal hemoglobinuria in contrast to those of normal subjects have been demonstrated to be abnormally subject to hemolysis in acidified human serum containing complement. It was thus possible that the patient's cells had an antigenic structure differing from normal cells. No significant difference was observed however, between the antigenic properties of red blood cells from Case 3 and from a normal subject of the same blood group when immune rabbit serums were produced against these erythrocytes and the antibody concentrations compared in cross-absorption experiments.

EXPERIMENTAL

Red blood cells from Case 3 and from a normal subject of the same blood group (I-AB) were obtained from

defibrinated blood, washed 3 times and made to 33 per cent suspensions in saline. Three albino rabbits were immunized with each suspension. Each animal received daily intravenous injections of 1, 2 and then 6 cc. of suspension for a total of 6 injections, followed in 10 days by a similar series of 6 daily injections. Eight days after the last injection, the antisera were tested for hemolysis and agglutination with red blood cells from each subject before and after cross-absorptions of each antiserum by the erythrocytes of the patient and control, respectively. For each absorption the rabbit antiserum, inactivated at 56 C. for from 30 to 60 minutes, and diluted 1 to 5 were treated with from 25 to 33 per cent by volume of washed packed erythrocytes for 30 minutes at 37 C.

All of the rabbit immune serums showed agglutination in high dilutions, varying from 1:2500 to 1:5000. Agglutination appeared in approximately equal dilutions with the washed red blood cells either from the patient or from the control. Cross-absorptions of each serum by the erythrocytes of the patient and of the control, respectively removed the agglutinins for the red cells of both subjects at the same rate and to the same extent. The unabsorbed rabbit antiserums produced complete hemolysis at the relatively low maximum dilution of 1:100. As in the agglutination tests, each rabbit antiserum possessed equal hemolytic activity for the erythrocytes of both subjects and cross-absorptions showed no selective removal of hemolysis by red cells of either subject.

4 Examination of patient's red blood cells for hemolytic antibody

Although no antigenic differences between patient's and normal erythrocytes were demonstrable in the above experiments, there remained the distinct possibility that the abnormal cells contained a hemolytic substance or antibody. It was conceivable, as one hypothesis, that the patient had developed a specific hemolytic antibody for his own red blood cells (as antigen), thus producing autoimmunization. In this event, a small amount of hemolytic antibody in the plasma might be completely absorbed by the antigen (red cells) leaving no demonstrable free antibody. Such "sensitized" cells might then hemolyze only in the presence of human complement. Should the amount of available antibody vary in concentration it is evident that the degree of "sensitization" or susceptibility to hemolysis of patient's red blood cells would be variable *in vivo* and might be observed to vary in a similar fashion *in vitro* under standardized experimental conditions. Accordingly the red blood cells from pa-

As shown in the data of Table VI, there was direct correlation between the removal of hemolytic activity and of complement, partially inactivated serum retained hemolytic potency roughly proportional to the complement concentration, completely inactivated serum showed no hemolytic activity. The combination of samples of human serum, inactivated respectively by ammonium hydroxide and by heat (presumably representing qualitatively all components of complement) restored the complement concentration to about one-fourth of its original titer, and for 1 of the 3 serums tested such recombination also restored in part the hemolytic activity. As shown in Table VII, the mixture containing the highest concentration of ammonium hydroxide-treated serum showed the greatest hemolytic power and complement concentration.

The addition of fresh guinea-pig serum always restored the hemolytic activity to human serum inactivated by ammonium hydroxide. Therefore, guinea-pig serum apparently supplied the fourth component of human serum essential for hemolysis but, as shown above, never supplied the ther-

TABLE VI

*Effect of ammonium hydroxide on complement and hemolytic activity **

Serum ammonium hydroxide mixtures incubated 1 hour at 37° C.†		Fresh human serum‡	Hemolysis† of red blood cells from Case 3	Hemolysis of normal red blood cells	Concentration of complement
Volume of normal ammonium hydroxide	Volume serum				
cc	cc.	Per cent of mixture			units per cc
		100	++	0	48
		100			
		Incubated 1 hour at 37.5° C			
0.375	1.65		++	0	48
0.250	1.87		0	0	0
0.187	2.00		0	0	0
0.125	2.10		trace	0	3
0.075	2.20		+	0	24

* Serum from normal subject treated with increasing amounts of ammonium hydroxide

† Approximate hemolysis

++ 10 per cent

+++ 20 per cent

++++ 30 per cent

‡ Serum pH finally adjusted to approximately 6.5 by adding hydrochloric acid

TABLE VII

*Mixture of inactivated serums restore hemolytic activity and complement **

Human serum treated with ammonium hydroxide†	Fresh serum‡	Serum‡ heated at 56° C for 5 minutes	Hemolysis of red blood cells from Case 3	Hemolysis of normal red blood cells	Concentration of complement
per cent of mixture	per cent of mixture	per cent of mixture	per cent	per cent	units per cc
	100		15	0	48
		100	0	0	0
100			0	0	0
	50	50	10	0	24
	20	80	2	0	6
80		20	4	0	12
50		50	3	0	12
20		80	±	0	6

* Serum from normal subject inactivated by ammonium hydroxide and by heat on recombination shows both hemolytic activity and complement

† See experimental

‡ Serum pH finally adjusted to approximately 6.5 by adding hydrochloric acid

molable components of heated human serum. The hemolytic activity was restored in part to ammonium hydroxide-inactivated human serum by whole guinea-pig serum and by heated guinea-pig serum but not by guinea-pig serum inactivated by ammonium hydroxide.

The combination of equal amounts of samples of serum treated with *zymum* and with *ammonium hydroxide*, respectively, restored approximately one-half of the complement concentration of the original serum, but did not restore the hemolytic power. Similar results, as referred to above, were obtained with mixtures of equal amounts of fresh and of heat-inactivated serum. Therefore, failure to observe hemolysis with either of these 2 different mixtures may have been due to a decreased *quantity* of complement and not to the lack of a particular component of complement.

EXPERIMENTAL

The procedure of inactivation of human serum by alkalinization to pH 9.0 or 9.1 with ammonium hydroxide, and the subsequent readjustment of the pH of the serum to approximately 6.5, were somewhat modified from the method of Gordon et al (23), in order to decrease the dilution of serum by the reagents. A mixture of 0.075 cc. of $\frac{1}{2}$ normal ammonium hydroxide and 0.884 cc. of serum was incubated at 37° C. for 75 minutes. The pH was then adjusted immediately to approximately 6.5 by the addition of 0.041 cc. of 1.0 normal hydrochloric acid and the hemolytic activity tested as usual. In 1 observation, using sodium hydroxide instead of ammonium

salt solution remaining after ether extraction (2 to 3 cc.) a saline suspension of the material containing lipoids left after evaporation of the ether and an 0.001 normal hydrochloric acid extract of the ether treated stroma. The action of these extracts on fresh normal and patient's cells was examined by incubating the following mixtures for 1 hour at 37° C. (1) Cells plus extract plus fresh acidified human serum (2) cells plus extract and (3) cells plus extract plus heated human serum. The supernatant fluids of mixtures (2) and (3) were then removed by centrifugation and the treated red cells tested for the presence of any "sensitization" by the addition of acidified serum in the usual manner.

Stroma from the erythrocytes of Case 3 was prepared by the method of Bennett and Schmidt and made to a 5 per cent suspension in saline. Equal quantities of suspensions of stroma and of normal erythrocyte (5 per cent) were mixed, centrifuged, and the salt removed. The cell stroma mixtures and plain stroma were tested with acidified serum.

5 Comparison with human isohemolysins

In the observations reported here, the possibility is considered that the patients red blood cells may be 'sensitized' by a hemolytic antibody requiring human complement for lysis. It has been demonstrated that certain animal serums, notably guinea pig serum do not restore hemolytic activity to heated human serum. Since a hemolytic antibody of human origin might be concerned, and since the complement requirements were quite specific, this hemolytic system was compared to the requirements of another human hemolytic system namely the hemolysis of group I-AB cells sensitized by the naturally occurring isohemolysins of serum from a normal subject of blood group IV-O.

Human cells when sensitized by human isohemolysins as described below, were hemolyzed to the same degree as cells from Case 3 when suspended in acidified human serum, thereby producing 2 hemolytic systems of human origin which were quantitatively comparable. Qualitatively however the following important differences were observed. The isohemolysin sensitized cells were hemolyzed during incubation for 1 hour at 37° C with guinea pig complement in dilutions of from 1/10 to 1/40 but unsensitized group I-AB cells or those from Case 3 were not hemolyzed under these conditions. Isohemolysin sensitized cells were significantly hemolyzed in fresh human complement when diluted 1/10 with saline or with heat inactivated human serum

whereas the cells from Case 3 showed no hemolysis in human serum diluted 1/25. Isohemolysin sensitized erythrocytes were always hemolyzed in unaltered human serum. The degree of hemolysis however, was increased significantly by acidification of serum with hydrochloric acid. Although the cells from Case 3 required acidification for hemolysis those of Cases 1, 2 and 5 were always hemolyzed in unacidified serum (2). Acidification of serum, therefore increased the degree of lysis for both hemolytic systems but was not necessarily an essential factor to either

EXPERIMENTAL

Sensitization of normal human erythrocytes was effected as follows. Separate samples of 0.5 cc. of a 5 per cent suspension of washed group I-AB erythrocytes from a normal subject were mixed with 0.5 cc. samples of normal group IV-O serum, which had been heated at 56° C. for 2 minutes. The mixtures were then incubated 1 hour at 37° C. and the packed (agglutinated) cells washed once with 5 cc. of saline. These packed cells, termed isohemolysin-sensitized red cells, together with similar samples of untreated I-AB cells, and of cells from Case 3 respectively were treated under identical conditions with group I-AB serum (as complement) acidified with hydrochloric acid, or with dilutions of unacidified guinea pig serum. In several observations the guinea pig serum was treated with an equal volume of packed human erythrocytes at 0° C. for 90 minutes to reduce the natural hemolysins of guinea pig serum for human red cells.

6 Susceptibility of patient's erythrocytes to hemolysis in 2 immunological systems

In the above observations no evidence was obtained that the patient's red blood cells contained any demonstrable hemolytic substance. It was essential, however, to secure information on the behavior of patient's red blood cells when exposed to other hemolytic systems of known immunological type. Such indirect evidence might have bearing on the nature of the abnormality of the patient's erythrocytes and the type of hemolytic activity involved. The susceptibility to hemolysis of patient's and normal cells was compared, therefore, in 2 immunological hemolytic systems one containing anti human rabbit serum and the other containing human isohemolysins. Complement was supplied either by human serum or guinea pig serum.

tients were examined, first, for differences in susceptibility to hemolysis, and, second, for the presence of hemolytic antibody which might be separated from the erythrocyte by procedures known to dissociate antigen-antibody combinations.

Comparisons of the susceptibility to hemolysis in vitro and in vivo of red blood cells from 4 patients showed striking correlation in individual patients between the susceptibility to hemolysis *in vitro* and the apparent degree of intravascular hemolysis as estimated by clinical criteria. This correlation reported previously (2) held for widely differing rates of blood destruction in different patients. Moreover, in the present prolonged study of Case 3, it was observed that such susceptibility *in vitro* varied materially at different periods in the same patient and corresponded with the apparent amount of hemolysis *in vivo*. This was demonstrated by determining at different times the percentage of hemolysis of the erythrocytes from Case 3 suspended in a standard amount of serum complement (fresh acidified serum from the same normal subject). The amount of hemolysis *in vitro* was then compared to the clinical evidence for hemolysis in the patient (degree of anemia, hemoglobinemia and hemoglobinuria).

Attempts to recover a hemolytic antibody combined with the patient's cells were carried out and the following extraction methods were employed: a modification of Locke and Hirsch's method (26) of ether extraction of erythrocyte stroma, treatment of erythrocytes with 10 per cent salt solution, using the technique of Heidelberger and Kendall (27), treatment with 10 per cent sucrose solution by the methods of Kosakai (28) and of Huntoon and Etris (29), extraction with 0.01 normal hydrochloric acid and sodium hydroxide, and, finally, direct use of erythrocyte stroma. Extracts of patient's or of normal red blood cells prepared by any one of the above methods were then tested for hemolytic activity with both patient's and normal cells, as described below.

In a series of preliminary experiments the efficiency of certain of these procedures was tested for recovery of the antibody from the red blood cells in 2 known antigen-antibody systems. In the first, in which sheep red blood cells were sen-

sitized with antishoop cell rabbit serum, 2 extraction procedures, those of Locke and Hirsch and of Kosakai, consistently resulted in the recovery of a small fraction of the hemolytic antibody employed. Using the technique of Heidelberger and Kendall, however, none of the antibody was recovered. In the second system, in which human type I-AB cells were sensitized by the naturally occurring isohemolysins of human serum of group IV-O, none of the hemolytic antibody was recovered by the Locke-Hirsch method. It was apparent, therefore, that even with known antigen-antibody systems, only a small fraction or none of the added hemolytic antibody was recoverable by the methods here employed.

When extracts of the red blood cells of Case 3 and of a normal subject, respectively, were made by any of the above procedures, no evidence was obtained for a hemolytic substance or antibody. Stroma derived from the erythrocytes of Case 3, like that of normal cells, appeared to have no effect on normal red blood cells when suspended together in acidified serum.

EXPERIMENTAL

Sensitization of red blood cells by known hemolytic antibodies was performed as follows. Sheep cells were sensitized with 2 units of antibody by adding 200 cc. of antishoop cell rabbit serum, diluted 1:2250, to 6 cc. of washed packed sheep red blood cells. After incubation for 30 minutes at 37° C the cells were washed 3 times and then extracted by the methods mentioned above. The extracts were tested quantitatively for the amount of antibody recovered. Normal human red blood cells were sensitized by adding 17 cc. (diluted to 35 cc.) of unheated group IV-O human serum (containing a high concentration of isohemolysins) to 35 cc. of a 5 per cent suspension of washed group I-AB erythrocytes. The mixture was incubated and washed, as described above, and the sensitized erythrocytes extracted.

An ether extract of erythrocyte stroma was made of the above sensitized red cells, as well as of the red cells from Case 3 and from a normal control, employing a modification of the method described by Locke and Hirsch. From 4 to 10 cc. of the washed packed erythrocytes were treated with 2 volumes of distilled water and any unhemolyzed cells removed by centrifugation. To the supernatant liquid 7 volumes of distilled water were added, and the mixture was saturated with CO₂ for 30 minutes as described by Bennett and Schmidt (30). The precipitated stroma was packed by centrifugation and then washed 4 times with distilled water, once with saline, and finally extracted 3 times with 15 cc. of ether. Three portions of the extract were tested, namely, the

*Cobra venom*⁴ dissolved in 1 per cent sodium chloride was added to fresh human serum, serum heated 5 minutes at 56 C., and to 1 per cent sodium chloride solutions to give concentrations of from 0.4 to 60 mgm. per 100 cc. The above preparations were mixed in equal quantities with 5 per cent suspensions of red cells from Case 3 and from a normal subject, incubated 1 hour at 37 C. and centrifuged for comparison of hemolysis.

DISCUSSION

In previous observations (1) (2) on 5 patients with paroxysmal nocturnal hemoglobinuria, it was demonstrated that their red blood cells were hemolyzed in their own fresh serums as well as in all fresh serums from normal subjects of compatible blood groups. The patients' serums were not lytic for normal cells. The essential abnormality in this disease thus appeared to reside in the erythrocytes. The hemolytic system, however, required the presence of fresh active human serum. The treatment of such serum by certain procedures, such as heating or the addition of anticoagulants and other salts, inhibited the hemolysis of patients' cells. These procedures are all known to inactivate or inhibit serum complement. Although acidification significantly increased the degree of hemolysis, it was not necessarily an indispensable requirement of the hemolytic system. The pH is known to be a modifying factor of many hemolytic systems (35) and possibly of certain antigen-antibody-complement reactions. These facts suggested that an immunological reaction might constitute the basis of the hemolytic mechanism in this disease. The reaction, accordingly, was investigated for other evidences of an antigen-antibody-complement type of hemolytic system.

Since hemolysis of patients' cells occurred in all compatible fresh human serums, at least 2 possibilities were considered to explain the hemolytic mechanism: first, that the abnormal erythrocytes were hemolyzed by a substance (antibody) present in all normal serums possibly requiring complement for lysis; and second, that the complement itself was the serum factor required for the hemolysis of red cells which were 'sensitized' by a hemolytic substance.

The first possibility that a hemolytic antibody was present in all serums, could be true only if the patients' cells were more susceptible to this antibody than normal cells. To support this hypothesis it should be possible to demonstrate certain characteristics of other human antibodies such as age distribution, specific or non specific absorption, association with heterophile antibody, isohemolysins, isoagglutinins or cold agglutinins. In the experiments reported above, however, there was no evidence obtained to indicate the presence of such an antibody in the serums from 5 patients and from the normal subjects studied.

Consideration of the second possibility must be divided into 2 aspects: first, that complement is the serum factor required for hemolysis and, second, that the red cells are sensitized by a hemolytic substance. Concerning serum complement it was observed that all procedures which reduced, inhibited or destroyed complement or any one of the individual components of complement, also reduced or eliminated the hemolytic activity of acidified serum for patients' erythrocytes. The hemolytic activity and the complement concentration were always partially restored to inactivated human serum by the addition of fresh human serum but a relatively large volume was required for these effects.

Certain remarkable features were observed with the use of animal serums as a source of complement. The addition of large concentrations of fresh undiluted guinea pig serum to fresh human serum usually increased its hemolytic activity for patients' erythrocytes without, however, producing hemolysis of normal cells. Similarly, guinea pig serum restored in part the hemolytic activity of human serum which had been only partially inactivated by heating, to the extent that in such heated serum, complement was definitely present but hemolytic activity was absent. Yet it is to be emphasized that neither fresh guinea pig serum nor the serums of 5 other animals restored hemolytic activity to human serum which had been completely inactivated by heat. This is perhaps not surprising since Hegedüs and Gracner (36) have shown that the thermolabile albumin component of complement is almost or entirely lacking in serums of the rabbit, dog, pig, ox and sheep and because Dingle *et al* (10) have demonstrated the failure of guinea pig serum to activate

⁴ Crystalline cobra venom was obtained through the courtesy of Doctor David I. Macht of Hinson, Wescott and Dunning Co., Baltimore, Md.

The *anti-human rabbit serums*, described in section 3, produced no greater hemolysis of the red cells from Case 3 than of those from a normal subject when guinea-pig serum was the source of complement. In the presence of human serum as complement, however, the erythrocytes of Case 3 showed a significantly greater susceptibility to hemolysis than normal cells. This was observed for the immune rabbit serums produced both by the patient's and by normal red blood cells. Under the conditions of the experiment, acidification did not increase the degree of hemolysis of patient's cells whether human or guinea-pig serum was the source of complement.

With *human isohemolysins* as antibody, the patient's erythrocytes were hemolyzed to a greater extent than normal erythrocytes when either human or guinea-pig serum supplied the complement.

EXPERIMENTAL

Anti-human rabbit serums were prepared as described in section 3. Serial dilutions were made of these heat-inactivated serums from 1:50 to 1:6400. Human red blood cells, which were derived from Case 3 and from the same normal subject whose cells were used in producing the immune rabbit serums, were washed 3 times and made into a 5 per cent suspension. Human complement was obtained from the normal subject of blood group I-AB and used either undiluted or diluted 1:2. Guinea-pig complement was employed as a 1:10 dilution of fresh guinea-pig serum. For acidification, $\frac{1}{2}$ normal hydrochloric acid was added in 5 per cent by volume to the undiluted serum used as complement. Equal volumes (0.5 cc.) of lytic agent, complement, and cell suspension, were mixed and incubated 1 hour at 37.5° C and the degree of hemolysis observed. There was no hemolysis of patient's cells by the 2 lytic agents or by the 2 complements when used alone. Using anti-human rabbit serum with human complement the patient's cells were hemolyzed significantly at a dilution of 1:3200, the normal cells at a dilution of 1:200, this occurred with both the homologous and heterologous rabbit serums. Using guinea-pig complement, however, there was no significant difference in the concentration necessary for hemolysis of both cells.

Human isohemolysins were obtained from group IV-O serum. The serum was heated at 56° C for 2 minutes to inactivate complement and serial dilutions were made up to 1:512. The red cells and complement were employed as described above. Using human isohemolysins with human complement, as well as with guinea-pig complement, the patient's cells were hemolyzed significantly at a dilution of 1:16, the normal cells at a dilution of 1:2.

7 Susceptibility of patient's erythrocytes to hemolysis in non-immunological systems

The susceptibility to hemolysis of patient's red blood cells was investigated in certain non-immunological hemolytic systems. As reported in previous publications (1, 2, 31, 32), the susceptibility to hemolysis by hypotonic salt solutions was normal for the red blood cells of the patients studied. In the experiments reported here, the susceptibility to hemolysis of erythrocytes of Case 3 was investigated in solutions of saponin, sodium taurocholate and of cobra venom.

The susceptibility of the patient's cells to hemolysis by *saponin* was the same as that of normal cells and considerably less than that of cells from a patient with untreated pernicious anemia (33). In 1 observation using *sodium taurocholate* as lytic agent, there was no demonstrable difference in the amount of hemolysis of patient's and of normal cells. *Cobra venom* produced direct lysis of washed human erythrocytes without requiring a complementary substance, as shown by Kyes (24). The degree of hemolysis varied directly with the concentration of venom and depended upon whether serum or saline was employed in the mixture. The cells of Case 3 were more susceptible to hemolysis than those of the control when suspended in mixtures of serum and cobra venom, whether the serum contained active or inactivated complement appeared to exert no influence. Conversely, the cells from Case 3 were less susceptible to hemolysis than control cells in mixtures of *saline* and cobra venom.

EXPERIMENTAL

Saponin was employed in the time dilution method of Ponder (34) and of Ponder and Rhoads (33), and in a modification of this procedure. In the modified method, samples were prepared of 1 cc. of saponin solution diluted from 1:1000 to 1:15,000 in 1 per cent sodium chloride. Two series of such dilutions were warmed to 37.5° C in a water bath and to each tube was added 1 cc. of a similarly warmed 2 per cent suspension of washed red cells in 1 per cent sodium chloride. After 10 and after 40 minutes incubation the separate series were centrifuged and the amounts of hemoglobin observed in the supernatant fluids.

Sodium Taurocholate (Merck) was employed in dilutions of from 1:1000 to 1:15,000 in 1 per cent sodium chloride, as described in the above modified method.

A third possibility which should be mentioned to explain the hemolytic system under investigation, is the hemolytic effect due to complement acting with agents other than immune bodies, as reviewed by Browning and Mackie (37). As shown by Landsteiner and co workers (38, 39), red cells sensitized by colloidal silicic acid are hemolyzed in the presence of complement. By analogy it is conceivable therefore that in paroxysmal nocturnal hemoglobinuria the patients' red blood cells may be conditioned by a substance other than an immune body and still require serum complement for hemolysis.

From the data presented here it is apparent that the abnormality involving the red blood cells of patients with paroxysmal nocturnal hemoglobinuria can not be defined. There is a distinct possibility that these cells are sensitized by a hemolytic substance conceivably an antibody. The origin of such a hypothetical antibody is not known but might result from autoimmunization. The serum factor essential for hemolysis is indistinguishable from serum complement. It is probable that increased acidity influences the degree of hemolysis by augmenting the activity of serum complement. The hemolytic system involved cannot be classified strictly as immunological in nature since there has been no demonstration of antigen or antibody. It is probable, however, that the hemolytic mechanism is an immunological system since complement or a complement-like substance is required for hemolysis and presumably because the red cells themselves show increased susceptibility to hemolysis in certain known immunological hemolytic systems.

CONCLUSIONS

1 In paroxysmal nocturnal hemoglobinuria the fundamental abnormality resided in the red blood cells which showed increased susceptibility to hemolysis when suspended in acidified plasma or serum from patients or from all normal subjects of compatible blood groups. The patients' red blood cells showed no increased susceptibility to hemolysis in the non immunological hemolytic systems consisting of saponin, sodium taurocholate or hypotonic solutions of sodium chloride. The patient's red blood cells showed increased susceptibility to hemolysis in the immunological hemolytic

systems consisting of anti human rabbit serum or human isohemolysins as antibody and of human serum as complement. No antigenic difference was demonstrated between patient's and normal erythrocytes when used to immunize rabbits. With the methods employed no hemolytic substance or antibody was demonstrated when patient's cells were treated by procedures known to dissociate antibody from antigen. The abnormality of patients' red cells has not been defined.

2 No hemolytic antibody or other abnormality was demonstrated in the patients' serum when compared to normal serum. The serum factor essential for hemolysis was closely associated with, if not indistinguishable from complement or alexin of human serum. For this hemolytic system the addition of guinea pig serum or the serum of certain other animals did not restore the thermostable components of human serum complement but guinea pig serum did restore the thermostable components.

3 The mechanism of hemolysis, probably immunological in nature, appeared to be that of an abnormal red blood cell which was hemolyzed in the presence of human complement, the degree of hemolysis varying directly with the susceptibility of the cell to lysis and with the acidity of the serum.

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BIBLIOGRAPHY

- 1 Ham, T. H., Chronic hemolytic anemia with paroxysmal nocturnal hemoglobinuria: study of mechanism of hemolysis in relation to acid base equilibrium. *New England J. Med.*, 1937, 217, 915.
- 2 Ham, T. H., Studies on destruction of red blood cells. I. Chronic hemolytic anemia with paroxysmal nocturnal hemoglobinuria: investigation of mechanism of hemolysis with observations on five cases. *Arch. Int. Med.* (In press).
- 3 Marchisava, E. and Nazari, A. Nuovo contributo allo studio degli itteri cronici emolitici. *Policlinico (sez. med.)* 1911, 18, 241.
- 4 Marchisava, E., Anemia emolitica con emosiderinuria perpetua. *Policlinico (sez. med.)* 1931, 38, 105.
- 5 Michel, F., Anemia (splenomegalia) emolitica con emoglobinuria-emosiderinuria tipo Marchisava. *Haematologica, I Arch.*, 1931, 12, 101.
- 6 Jordan, F. L. J. Etudes sur l'hémoglobinurie. *Acta Med. Scandinav.*, 1938, 95, 319.

certain antigen-antibody reactions. There was evidence, therefore, that the hemolytic mechanism investigated here specifically required the thermolabile components of complement derived from human serum. On the contrary, guinea-pig serum in most instances restored in part the hemolytic activity to human serums in which the *thermostable* components of complement were inactivated by zymine absorption or by treatment with ammonium hydroxide.

There were, however, several inconsistencies which appeared to prevent full acceptance of the identity of the serum factor essential for hemolysis to be serum complement. Three of these, *i.e.*, increased hemolysis with acidification, failure of fresh animal serums to reactivate heated serum, and the limiting factor of dilution before complement activity was lost, have been discussed previously and may not be valid objections. Removal of hemolytic activity and complement by untreated influenza bacilli was probably due to specific anti-*H influenza* antibodies present in adult human serums (11). No explanation has been found, however, for the decrease of hemolytic activity in lyophilized serums without appreciable decrease in complement, nor for the failure to demonstrate specific utilization or fixation of complement even after hemolysis of the patient's cells had taken place repeatedly in the same serum. One explanation may lie in the extreme sensitivity of the sheep red blood cell hemolytic system to small amounts or to particular components of complement, thereby masking slight quantitative differences essential to the hemolytic mechanism in paroxysmal nocturnal hemoglobinuria. An explanation of the failure to demonstrate complement fixation may be found in the analogy that red blood cells sensitized with silicic acid, requiring complement for lysis, have very little capacity for fixing complement (37). Although serum complement cannot be identified directly, it appeared from this evidence that the serum factor in this hemolytic mechanism corresponded in its general behavior to complement or to a complement-like substance. It did not appear likely, however, that complement itself was the hemolytic agent.

The other aspect of the second possibility referred to above, namely, that patients' red blood cells might be sensitized by a hemolytic substance, was further examined. It was observed that the susceptibility to hemolysis of patients' red blood

cells varied significantly from patient to patient and in the same patient. Such wide quantitative fluctuation was at least compatible with differing degrees of sensitization of red cells by a hemolytic antibody, which conceivably might result from autoimmunization. Nevertheless, no hemolytic substance associated with patients' erythrocytes could be demonstrated by a variety of procedures known to dissociate antigen-antibody combinations. It must be emphasized, however, that the methods as employed here may have been inadequate since from red blood cells sensitized to known antibodies only a small fraction or none of the absorbed antibody was recovered by certain of these extraction procedures. It was a further possibility that an abnormal constituent of the patient's red blood cells might be detectable by its *antigenic properties*. No antigenic difference between patient's and normal erythrocytes was demonstrable, however, when these cells were used to immunize rabbits and cross-absorption experiments were performed with the antiserums. It was observed further that the hemolytic system consisting of patient's cells and serums differed in the important feature of complement requirements from the hemolytic system consisting of human red cells sensitized with human isohemolysins and of complement derived from human or guinea pig serum. This represents indirect evidence that, if the patients' red blood cells are sensitized by an antibody, the antibody differs in behavior from human isohemolysins.

As a further immunological study, the patient's cells were subjected to hemolysis in 2 hemolytic systems *known* to be of the antigen-antibody-complement type, employing as hemolytic antibodies, first, anti-human rabbit serum, and, second, the isohemolysins of human serum. In both systems, when human complement was employed, the patient's cells showed greater susceptibility to hemolysis than did normal human cells. This was suggestive but indirect evidence that the abnormality of patients' red blood cells might be related to an immunological type of reaction. Such a possibility was further emphasized by the observations that the patients' red blood cells were not abnormally susceptible to hemolysis in the non-immunological hemolytic systems consisting of hypotonic salt solutions, saponin or sodium taurocholate.

EFFECT OF PITRESSIN IN CIRCULATORY COLLAPSE INDUCED BY SODIUM NITRITE

By EUGENE A. STEAD, JR., PAUL KUNKEL, AND SOMA WEISS

(From the Thorndike Memorial Laboratory Second and Fourth Medical Services (Harvard), Boston City Hospital and the Department of Medicine Harvard Medical School Boston)

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Pitressin is frequently used in the treatment of circulatory collapse. Evaluation of its usefulness has been hindered by the difficulty of interpreting symptoms and of obtaining circulatory measurements in critically ill patients. Previous studies on normal subjects in the upright position (1) (2) have demonstrated that sodium nitrite produces a type of collapse, often ending in syncope, which is similar in its circulatory manifestations to certain types of clinical shock. The symptoms of this collapse are accompanied by a fall in arterial pressure and by a decrease in pulse pressure. There is an increase in arteriolar tone, but a decrease in venous tone. The blood flow in the hand as indicated by plethysmographic studies shows no marked changes during the early stage of collapse because of compensatory changes, later however, there is a progressive fall in blood flow, which frequently drops to zero as syncope develops. The syncope is the result of progressive cerebral anoxia which develops when the venous return to the heart and the cardiac output are decreased because of the blood pooled in the veins of the dependent portions of the body. This type of collapse, which could be produced under controlled conditions was ideal for study of the action of pitressin. Similar studies on the effect of epinephrin have previously been reported (3)

METHODS

The subjects rested on a tilting table in the horizontal position for at least 30 minutes after all apparatus had been adjusted and before any observations were made. The heart rate was counted by arterial palpation or by precordial auscultation. Arterial pressure was determined in the arm at heart level by the auscultatory method, using a mercury manometer. The blood flow in the hand was measured by Freeman's modification (4) of the plethysmographic method of Hewlett and Van Zwaluwenburg (5). The increases in hand volume caused by raising the venous pressure from the resting level to 20, 30 and 40 mm. Hg were used to indicate the venous tone or venous distensibility calculated as cubic

centimeters per liter of hand, according to the method described by Capps (6). The water bath in which the right hand was immersed was maintained at 32° or at 37° C. The vessels of the left hand were dilated by keeping the temperature of the water bath at from 43° to 45° C. The venous tone in the hand at 43° C. was not computed, since in some subjects the measurement has been found to be unsatisfactory at temperatures above 40° C.

The experiments consisted of the following procedures carried out on the same subject. After repeated control measurements had been obtained observations were made on different days on the effects of (1) elevation of the subject to the upright position (75 degrees) for 30 minutes followed by return to the horizontal position (2) intramuscular administration of 0.5 or 1.0 cc. of pitressin, followed in 10 to 18 minutes by elevation to the upright position for 30 minutes if collapse did not occur and return to the horizontal position (3) oral administration of 0.12 or 0.18 gram (2 or 3 grains) of sodium nitrite to the subject in the horizontal position, followed in 25 minutes by elevation to the upright position (4) administration in the horizontal position of the same dose of sodium nitrite and, in 15 minutes, the same dose of pitressin, followed in 10 minutes by elevation of the subject to the upright position. The subject was urged to remain motionless while in the upright position. At the height of the collapse, which was associated with syncope, the subject was promptly returned to the horizontal position and observations were continued for at least 30 minutes. Studies on the effect of pitressin (7) and of sodium nitrite (1) (2) in the horizontal position have been reported.

Six normal young adults served as subjects. Four of them had a complete series of experiments, as outlined above. After the injection of pitressin, 2 subjects developed syncope when they were tilted to 75 degrees, therefore they were not given sodium nitrite.

RESULTS

The effect of the intramuscular administration of 1 cc. of pitressin followed by tilting of the subjects to the upright position was studied in 6 subjects. Four subjects stood without difficulty. 2, however, fainted. Figure 1 shows the usual circulatory adjustments which occurred in the group of subjects who stood without difficulty. In subjects in the horizontal position pitressin produced

- 7 Osborn, T W B, Complement or Alexin. Oxford University Press, London, 1937
- 8 Noguchi, H., Serum Diagnosis of Syphilis Lippincott Co, Philadelphia, 1910
- 9 Bing, F C., and Baker, R. W., Determination of hemoglobin in minute amounts of blood by Wu's method. *J Biol Chem*, 1931, 92, 589
- 10 Dingle, J H, Fothergill, L D., and Chandler, C. A., Studies on *Haemophilus influenzae*, failure of complement of some animal species, notably guinea pig, to activate bactericidal function of sera of certain other species *J Immunol*, 1938, 34, 357
- 11 Fothergill, L D., and Wright, J., Influenzal meningitis, relation of age incidence to bactericidal power of blood against casual organism *J Immunol*, 1933, 24, 273
- 12 Ward, H K., and Enders, J F., Analysis of opsonic and tropic action of normal and immune sera based on experiments with pneumococcus *J Exper Med.*, 1933, 57, 527
- 13 Dacie, J V., Israels, M C. G., and Wilkinson, J F., Paroxysmal nocturnal haemoglobinuria of the Marchiafava type *Lancet*, 1938, 1, 479
- 14 Thannhauser, S J., and Setz, P., Studies on animal lipids, method for quantitative determination of diaminophosphatide in organs and fluids, application to stromata of red blood cells and serum. *J Biol Chem*, 1936, 116, 533
- 15 Van den Bergh, A. A. H., Ictere hemolytique avec crises hemoglobinuriques Fragilite globulaire. *Revue de Med.*, 1911, 31, 63
- 16 Flosdorf, E. W., and Mudd, S., Procedure and apparatus for preservation in "lyophile" form of serum and other biological substances *J Immunol.*, 1935, 29, 389
- 17 Strong, P S., and Culbertson, J T., Filtrability of components of alexin. *J Hyg*, 1934, 34, 522
18. Wright, H D., and MacCallum, P., Effect of electrolytes on hemolysis *J Path. and Bacteriol.*, 1922, 25, 316
- 19 Wadsworth, A., Maltaner, F., and Maltaner, E., Studies on activity of cephalin as it relates to coagulative and complementary properties of blood. *J Immunol.*, 1936, 30, 417
- 20 Wadsworth, A., Maltaner, F., and Maltaner, E., Inhibition of complementary activity by anticoagulants *J Immunol.*, 1937, 33, 297
- 21 Liefmann, H., Ueber den Mechanismus der Sero-reaktion der Lues *München. med. Wchnschr*, 1909, 56, 2097
- 22 Whitehead, H R., Gordon, J., and Wormall, A., The "third component" or heat-stable factor of complement. *Biochem J*, 1925, 19, 618
- 23 Gordon, J E., Whitehead, H R., and Wormall, A., Action of ammonia on complement, fourth component. *Biochem J*, 1926, 20, 1028
- 24 Kyes, P., Venom hemolysis *J Infect. Dis*, 1910, 7, 181
- 25 Browning, C H., and Mackie, T J., Relationship of complementing action of fresh serum along with immune body to its haemolytic effect with cobra venom, contribution on structure of complement. *Zeitsch f Immunitätsforsch.*, 1913, 17, 1
- 26 Locke, A., and Hirsch, E. F., Isolation of substances with immune properties *J Infect. Dis.*, 1925, 37, 449
- 27 Heidelberger, M., and Kendall, F E., Quantitative studies on antibody purification, dissociation of precipitates formed by pneumococcus specific polysaccharides and homologous antibodies *J Exper Med.*, 1936, 64, 161
- 28 Kosakai, M., Isolation, purification and concentration of immune bodies study of immune hemolysis *J Immunol*, 1918, 3, 109
- 29 Huntoon, F M., and Etris, S., Antibody studies, recovery of antibody from sensitized antigens technic. *J Immunol*, 1921, 6, 123
- 30 Bennett, C B., and Schmidt, C L. A., On red cell globulin. *J Immunol.*, 1919, 4, 29
- 31 Witts, L J., Paroxysmal haemoglobinurias *Lancet*, 1936, 2, 115
32. Hamburger, L. P., and Bernstein, A., Chronic hemolytic anemia with paroxysmal nocturnal hemoglobinuria. *Am. J M Sc.*, 1936, 192, 301
- 33 Ponder, E., and Rhoads, C P., Red cell resistance to lysins in pernicious anemia. *Proc. Soc. Exp Biol and Med.*, 1938, 38, 540
- 34 Ponder, E., Protoplasma. Monographien. The Mammalian Red Cell and the Properties of Haemolytic Systems Verlag von Gebrüder Borntraeger, Berlin, 1934
- 35 Walbum, L E., Importance of hydrogen ion concentration in hemolysis by lysins of anaerobic bacteria. *J Path., and Bact.*, 1938, 46, 85
- 36 Hegedüs, A., and Greiner, H., Quantitative Bestimmung der Komplementbestandteile. *Ztschr f Immunitätsforsch. u. exper Therap*, 1938, 92, 1
- 37 Browning, C H., and Mackie, T J., Immunochemical Studies Constable and Co, London, 1925
- 38 Landsteiner, K., und Jagic, N., Ueber Reaktionen anorganischer Kolloide und Immunkörperreaktionen. *München med Wchnschr*, 1904, 51, 1185
- 39 Landsteiner, K., and Rock, H., Untersuchungen über Komplementwirkung Hämolyse durch Kieselsäure und Komplement. *Ztschr f Immunitätsforsch.*, 1912, 14, 14

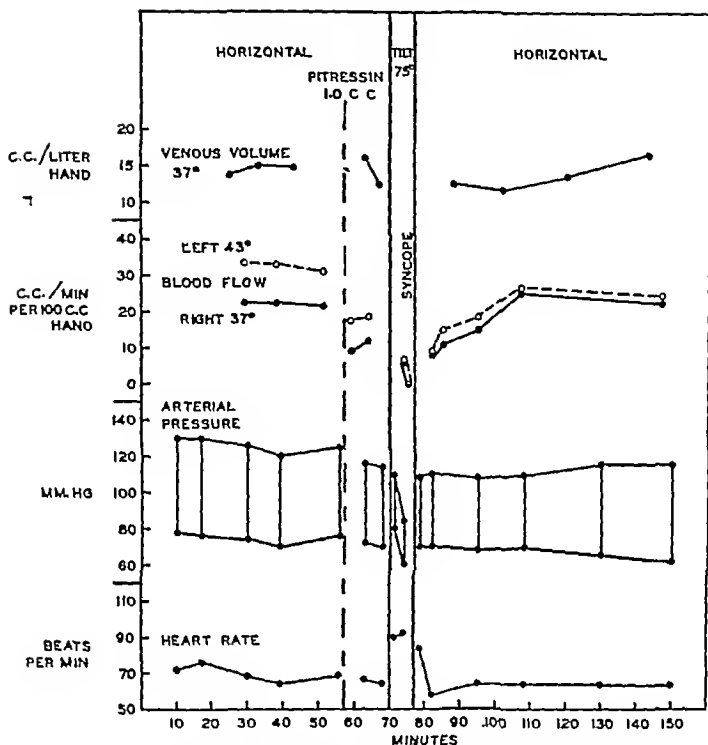


FIG. 2. COLLAPSE ENDING IN SYNCOPE INDUCED IN A NORMAL MALE SUBJECT L. R., AGE 26 BY 1 CC. OF PITRESSIN INTRAMUSCULARLY FOLLOWED IN 13 MINUTES BY TILTING TO AN ANGLE OF 75 DEGREES ABOVE THE HORIZONTAL

pallor and sweating but with normal circulatory adjustments namely a moderate drop in systolic and pulse pressures a slight increase in pulse rate and venous tone and a moderate diminution in blood flow to both the warm and cool hands. These mild symptoms disappeared immediately when the subject assumed the horizontal position. The administration of pitressin to the subject in the horizontal position produced marked pallor and a slight drop in blood pressure. The blood flow in both hands was decreased by 50 per cent. After the subject was tilted to 75 degrees ashen pallor, weakness, nausea and dilated pupils developed in 7 minutes syncope occurred. During the period

of collapse there was a marked fall in systolic pressure and a lesser fall in diastolic pressure, resulting in a great decrease in pulse pressure. The pulse rate reached 90 per minute and at the time of syncope the blood flow in both hands had fallen to zero. When the subject was returned to the horizontal position the symptoms rapidly disappeared though the blood flow to the hands remained well below control levels for the next 30 minutes.

In 2 normal subjects collapse ending in syncope occurred as the result of tilting to 75 degrees after the ingestion of 0.18 gram (3 grains) of sodium nitrite. In one of these subjects syncope occurred 14 minutes after tilting but, when pitressin was

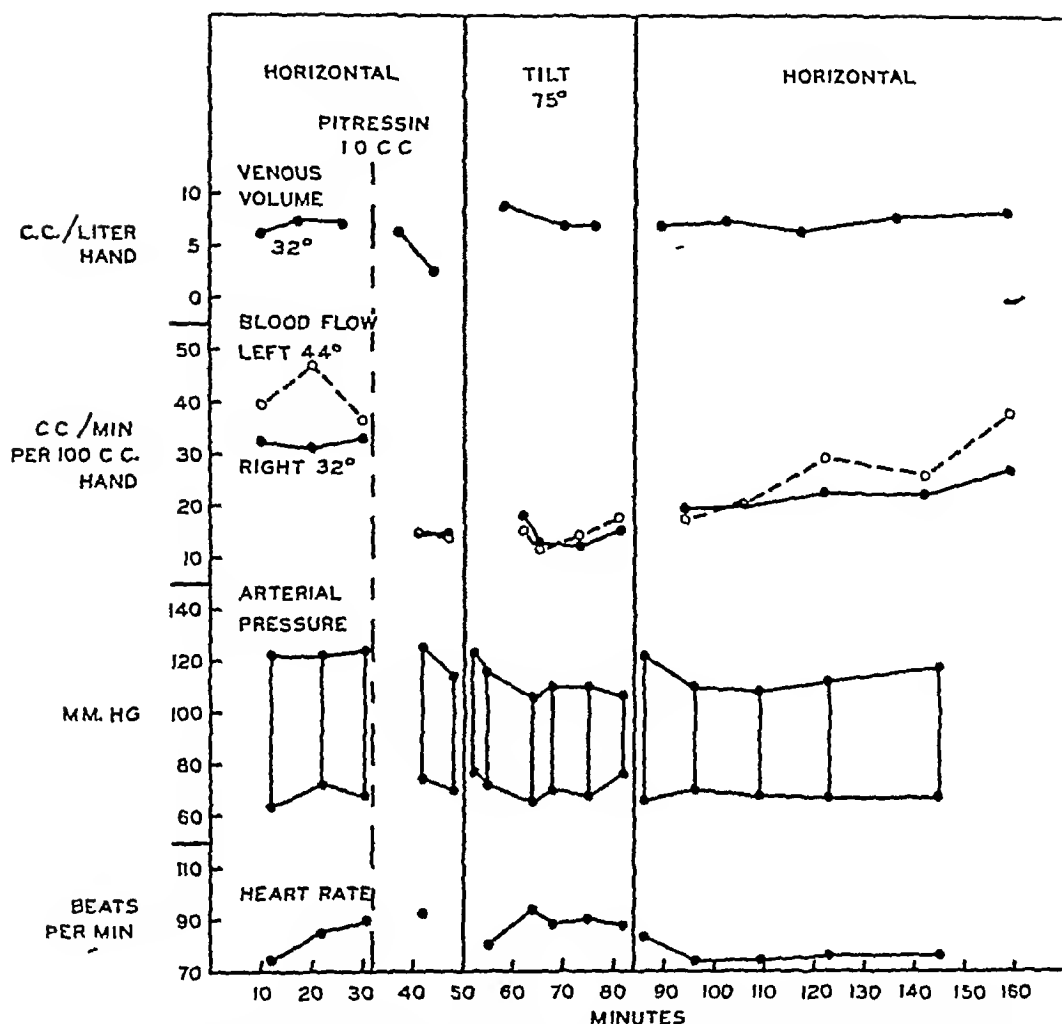


FIG 1 THE EFFECT IN A NORMAL MALE SUBJECT, E S, AGE 29, OF 1 CC. OF PITRESSIN INTRAMUSCULARLY FOLLOWED IN 18 MINUTES BY TILTING TO 75 DEGREES

dominal cramps, ashen pallor and a marked decrease in blood flow in the hand, both at 32° and at 44° C. The venous tone in the hand showed no consistent change. Pitressin had no constant effect on the heart rate and blood pressure. The most common findings were a slight elevation of the diastolic pressure, a slight fall in the systolic pressure and an unaltered heart rate. When the subject was raised to the upright position no significant changes in the heart rate or arterial pressure occurred. The systolic pressure was frequently lowered and the pulse pressure slightly narrowed. The blood flow in both hands remained very low. No new symptoms developed. The abdominal cramps, however, were usually relieved when the subject was placed in the upright

position. At the end of 30 minutes the subject was returned to the horizontal position. In the next hour the blood flow in the hands gradually returned to normal.

The combination of the intramuscular injection of pitressin and of tilting the subject to an angle of 75 degrees produced syncope in 2 normal young adults, in 1 within 7 minutes after tilting, in the other within 2 minutes. These subjects had previously stood motionless for periods of 30 minutes without syncope. Figure 2 shows the reactions of a normal 26-year-old male, L. R., to tilting to 75 degrees 12 minutes after 1 cc of pitressin was administered intramuscularly. Without any medication this subject had previously stood for 30 minutes with slight fullness in the epigastrium,

EFFECT OF PAREDROLINOL (α -N-DIMETHYL-p-HYDROXYPHENETHYLAMINE) ON SODIUM NITRITE COLLAPSE AND ON CLINICAL SHOCK

By PAUL KUNKEL, EUGENE A. STEAD JR., AND SOMA WEISS

(From the Thorndike Memorial Laboratory Second and Fourth Medical Services (Harvard) Boston City Hospital and the Department of Medicine Harvard Medical School Boston)

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Previous studies (1) (2) have demonstrated that circulatory collapse similar to that often observed in disease and suitable for experimental study is induced in normal subjects in the upright position by administering sodium nitrite. This collapse results from decreased venous tone, which causes pooling of blood in the veins and venules of the dependent portions of the body. Consequently the cardiac output decreases and with the ensuing cerebral anoxia, syncope usually occurs. Epinephrin and pitressin have been found to be ineffective in preventing this type of collapse and syncope (3) (4). Indeed in a number of cases pitressin aggravated the circulatory collapse. Epinephrin causes a definite increase in venous tone (3) but at the same time it necessitates a greater cardiac output because of the increased metabolic rate and widespread chemical changes in certain tissues. Thus while in sodium nitrite collapse epinephrin may increase the venous return to the heart to some extent it does not cause enough increase to compensate for the augmented tissue requirements. Pitressin does not affect the venous tone and therefore has no favorable action in the type of collapse investigated. In addition both these drugs produce arteriolar constriction in certain organs (3) (4). This action is unfavorable for as venous pooling occurs, compensatory reflex arteriolar constriction takes place. An increase in this arteriolar constriction by drugs, therefore, further reduces the tissue blood flow and accentuates tissue anoxia.

Since in sodium nitrite collapse venous pooling and reflex arteriolar constriction occur the ideal drug for the prevention of such collapse should produce constriction of the veins and venous reservoirs without arteriolar constriction or possibly with arteriolar dilatation. Such a substance should not cause increased oxygen consumption or widespread chemical changes in the body. From studies of normal subjects it was found that

paredrolinol¹ (α -N-dimethyl p-hydroxyphenethylamine) fulfilled several of these requirements (5). Therefore its action was investigated in the collapse induced by sodium nitrite and in that accompanying postural hypotension and acute infectious disease.

METHODS

In 7 subjects with normal cardiovascular systems collapse ending in syncope was induced by the administration of sodium nitrite. The heart rate was counted by arterial palpation or by precordial auscultation. Arterial pressure was measured in the arm at heart level by the auscultatory method, using a mercury manometer. The blood flow in the hand was determined by Freeman's modification (6) of the plethysmographic method of Hewlett and Van Zwailenburgh (7) and the venous tone by the plethysmographic method of Capps (8). The subject rested on a tilting table in the horizontal position for at least 30 minutes after all apparatus had been adjusted and the water in the plethysmograph had reached the desired temperature.

The following experiments were carried out in each of the 7 subjects on different days. (1) The oral administration of 0.18 gram (3 grains) of sodium nitrite was followed in 15 minutes by the intramuscular injection of sterile saline and in 25 minutes by tilting the subject to an angle of 75 degrees above the horizontal. When collapse and syncope developed the subject was immediately returned to the horizontal position. (2) The oral administration of 0.18 gram (3 grains) of sodium nitrite was followed in 15 minutes by the intramuscular injection of from 25 to 45 mgm of paredrolinol and in 25 minutes by tilting to the upright position (75 degrees). Unless collapse ending in syncope occurred the subjects were allowed to stand motionless for 30 minutes and then returned to the horizontal position. If the subject did not develop collapse when paredrolinol was used, the experiment was repeated with sodium nitrite followed by the injection of sterile saline in order to ascertain that the person was still sensitive to nitrite. The order of the experiments was varied and the subjects were not told what medication they were receiving. In addition to these experiments on normal subjects the effect of paredrolinol on the arterial pressure, the heart rate and the

¹ The paredrolinol sulphate used in this investigation was supplied through the courtesy of the Smith, Kline & French Laboratories, Philadelphia.

here reported, however, indicate that pitressin would not have a favorable action, and, indeed, it may well be harmful, in the very common type of collapse associated with cold, ashen gray skin, with small, thready, rapid pulse, with decreased venous return to the heart and with arteriolar constriction, as manifested by a fairly well-maintained diastolic pressure, low pulse pressure and decreased blood flow in the tissues

SUMMARY AND CONCLUSIONS

1 Pitressin in man was ineffective in experimental collapse induced by sodium nitrite and tilting because it did not cause an increase in venous tone, and because the arteriolar constriction produced by the drug tended further to reduce tissue blood flow

2 The intramuscular injection of 1 cc. of pitressin with subsequent tilting of the subject to the upright position produced collapse ending in syncope in 2 of the 6 normal subjects tested

3 In 2 subjects in whom collapse was induced in the upright position by the administration of sodium nitrite, pitressin did not prevent the development of collapse and in 1 of these it hastened it. In 1 subject, in whom neither sodium nitrite nor pitressin in the upright position produced collapse, sodium nitrite followed by pitressin induced profound collapse ending in syncope. In still another subject neither sodium nitrite nor pitressin, singly or combined, produced syncope

4 Pitressin, given in doses of 0.5 or 1 cc. to normal subjects in the horizontal position, produced abdominal cramps, ashen pallor and a marked decrease in blood flow both in the hand at 32° and that at 43° C. It produced no change in venous tone in the hand, heart rate or arterial pressure.

5 Pitressin slowed the blood flow in the hand to such a degree that water at a temperature of from 43° to 45° C. felt distinctly uncomfortable,

and in 1 case caused the skin to be blistered at 45° C

6 Sodium nitrite produced circulatory collapse and syncope in the upright position in about 50 per cent of a large group of subjects tested. No criteria have been developed to predict the postural response of any given person to the administration of sodium nitrite.

This investigation was carried out with the technical assistance of Miss Sophia M. Simmons, S.B.

BIBLIOGRAPHY

- 1 Weiss, S., Wilkins, R. W., and Haynes, F. W., The nature of circulatory collapse induced by sodium nitrite. *J. Clin. Invest.*, 1937, 16, 73
- 2 Wilkins, R. W., Haynes, F. W., and Weiss, S., The role of the venous system in circulatory collapse induced by sodium nitrite. *J. Clin. Invest.*, 1937, 16, 85
- 3 Wilkins, R. W., Weiss, S., and Haynes, F. W., The effect of epinephrin in circulatory collapse induced by sodium nitrite. *J. Clin. Invest.*, 1938, 17, 41
- 4 Freeman, N. E., The effect of temperature on the rate of blood flow in the normal and in the sympathectomized hand. *Am. J. Physiol.*, 1935, 113, 384
- 5 Hewlett, A. W., and Van Zwaluwenburg, J. G., The rate of blood flow in the arm. *Heart*, 1909-10, 1, 87
- 6 Capps, R. B., A method for measuring tone and reflex constriction of the capillaries, venules and veins of the human hand with the results in normal and diseased states. *J. Clin. Invest.*, 1936, 15, 229
- 7 Kunkel, P., Stead, E. A., Jr., and Weiss, S., Blood flow and vasomotor reactions in the hand, forearm, foot, and calf in response to physical and chemical stimuli. *J. Clin. Invest.*, 1939, 18, 225
- 8 Erlanger, J., Gesell, R., and Gasser, H. S., Studies in secondary traumatic shock. I. The circulation in shock after abdominal injuries. *Am. J. Physiol.*, 1919, 49, 90
- 9 Freeman, N. E., Shaw, J. L., and Snyder, J. C., The peripheral blood flow in surgical shock. *J. Clin. Invest.*, 1936, 15, 651
- 10 Weiss, S., and Wilkins, R. W., Syncope, collapse and shock: their medical significance and their treatment. *M. Clin. North America*, 1937, 21, 481

demonstrated that the venous tone is increased and that the venous pressure rises by from 30 to 40 mm. of water. Thus the action of the drug on the veins (Figure 3) is antagonistic to that of sodium nitrite which has been shown to decrease the venous tone and to produce hypotension and collapse in the upright position by venous pooling in the dependent portions of the body. Fortunately paredrinol has no unfavorable side reactions. It does not produce pain. It does not increase metabolism, as does epinephrin and thus create a need for greater cardiac output. It does not cause marked arteriolar constriction.

Epinephrin (3) has likewise been shown to increase the venous tone (Figure 3). This effect, which should be useful in collapse caused by venous pooling, is however, overbalanced by the rise in metabolism and by chemical changes initiated in the muscles and liver which probably create a need for a greater cardiac output. Pitressin does not increase the venous tone (Figure 3) and therefore is not useful in this type of collapse. In addition, both epinephrin and pitressin cause arteriolar constriction in certain organs. This is an unfavorable action because the arterioles have already undergone reflex contraction

due to the fall in blood pressure and the additional arteriolar constriction induced by the drug reduces the already diminished blood supply to the tissue.

After the administration of sodium nitrite and paredrinol the heart rate always becomes very rapid when the subject is tilted to the upright position. The slow heart rate usually present in normal subjects after the injection of paredrinol results from vagal inhibition from stimuli passing through the carotid sinus and aortic nerves. The heart rate becomes rapid, therefore, if the vagal influence is eliminated by atropine (8), or if the stimulus to the carotid sinus and aortic nerve endings is removed by a fall in blood pressure, as is the case in nitrite collapse. Similar changes are present in clinical collapse. In these cases the heart rate usually increases when paredrinol is given probably because the pressure does not rise high enough to produce vagal inhibition by stimulation of the sensitive vascular areas. Experiments with atropine have shown that paredrinol causes an increase in heart rate when vagal influence is eliminated.

In 7 of the 10 patients in severe clinical collapse, the injection of paredrinol resulted in a rise

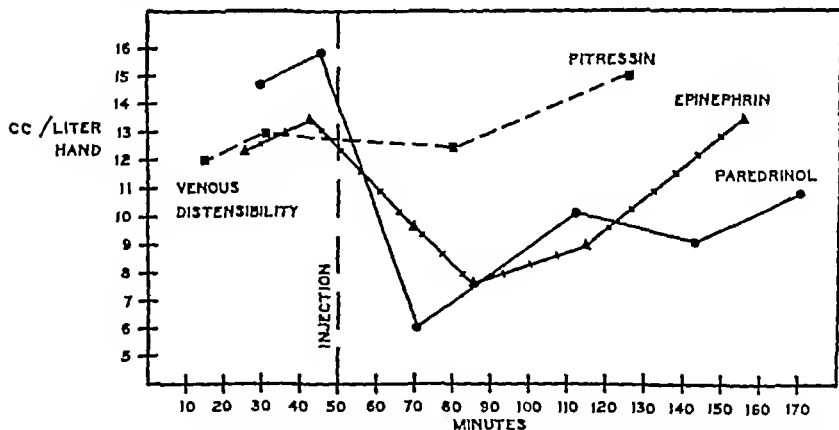


FIG. 3 THE EFFECT OF THE INJECTION OF PAREDINOL, EPINEPHRIN AND PITRESSIN ON THE VENOUS TONE IN THE SYMPLECTOMIZED (PREGANGLIONIC) HAND

An occluding pressure of 30 mm. was applied to the wrist and the resulting increase in hand volume was measured. A decrease in venous distensibility indicates an increase in venous tone.

standing After the intramuscular injection of from 20 to 35 mgm of paredrinol, these subjects stood for 30 minutes without developing any symptoms of collapse

Clinical shock Paredrinol was given either intramuscularly or intravenously in doses of from 15 to 50 mgm to 10 patients in severe medical shock Eight had acute infectious diseases, 2 had milary tuberculosis In addition to a low arterial pressure and a small pulse pressure, the majority of the patients had cold extremities, which indicated a slow peripheral blood flow In 7 of the 10 patients a rise in arterial pressure was produced, in 3 no rise occurred In only 2 of the patients did the arterial pressure rise above the normal level The response of these cases in clinical collapse to paredrinol differed from that in normal subjects in at least 3 ways

(1) Much larger doses of the drug (up to 50 mgm intravenously) were required to produce a rise in arterial pressure, and the effect of the drug lasted only from 15 to 30 minutes, while in the normal subject the effect of an intramuscular injection of 25 mgm of paredrinol usually lasted 60 minutes, (2) the heart rate was as a rule greatly increased, while in normal subjects it was usually decreased, (3) repeated injections of the same amount of the drug a short time after the arterial pressure had fallen to the original level produced less response each time, until in most cases large doses no longer had any effect on the arterial pressure In 3 of these subjects in shock the blood flow in the dilated hand was measured In 2 of these cases in which paredrinol did not produce a rise in arterial pressure, the blood flow in the hand decreased and the extremities became cold, in one in which paredrinol produced a definite rise in blood pressure the blood flow in the hand did not definitely increase In the third subject the usual spontaneous fluctuations in vasomotor tone and the reflex vasoconstrictor responses to sensory stimuli were absent before the administration of paredrinol because of the presence of shock Paredrinol did not restore these functions although it raised the blood pressure

Clinical improvement was definitely established by the administration of paredrinol in only 2 of the 10 cases with circulatory collapse In one of these patients collapse followed the adminis-

tration of horse serum The second subject, who had an acute streptococcal infection of the throat, collapsed when she was placed in Fowler's (semi-recumbent) position After this patient became afebrile, her postural reactions were investigated When she was tilted to an angle of 75 degrees syncope developed in 3 minutes, whereupon she was returned to the horizontal position She was then given 25 mgm of paredrinol intramuscularly and the arterial pressure rose from 100 mm systolic and 60 mm diastolic to 160 mm systolic and 80 mm diastolic The heart rate dropped from 70 to 63 beats per minute On being tilted to an angle of 75 degrees the patient became paler but stood without difficulty for 15 minutes, when she developed nausea and was returned to the horizontal position At that time the blood flow in the hand was fairly well maintained The arterial pressure was 100 mm systolic and 70 mm diastolic, the heart rate was 140 Thus in this patient both spontaneous collapse in the course of a streptococcus infection and subsequently induced collapse and syncope were improved by paredrinol

DISCUSSION

The effects in normal subjects of the intramuscular administration of paredrinol have been reported (5) The subjects experienced no symptoms except palpitation The apex impulse became more forceful, arterial pulsations in the neck more prominent and the heart sounds increased in intensity The intramuscular injection of 25 mgm of the drug produced in 10 normal subjects an average arterial pressure of 173 mm systolic and 92 mm diastolic The pulse rate usually fell The venous pressure was elevated by from 30 to 40 mm of water The venous tone was also increased The blood flow in the hand at 43° C became slower, but that in the foot, forearm and calf was unchanged The cardiac output, circulation time and metabolic rate showed no significant changes

The favorable effect of paredrinol on the collapse induced by sodium nitrite and posture results mainly from the action of the drug on the venous system Experiments on animals have shown that paredrinol causes an increase in venous tone and an emptying out of the venous reservoirs Studies on normal subjects have

BLOOD CHEMICAL CHANGES IN BOECK'S SARCOID WITH PARTICULAR REFERENCE TO PROTEIN CALCIUM AND PHOSPHATASE VALUES

By GEORGE T. HARRELL AND SARA FISHER

(From the Departments of Medicine and Biochemistry, Duke University School of Medicine and Duke Hospital, Durham, North Carolina)

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Definite alterations in the concentration of various chemical constituents of the blood occur in Boeck's sarcoid. This rare disease of unknown etiology is characterized by a marked proliferation of epithelioid cells arranged in the form of tubercles and usually runs a benign course of long duration. The clinical types of the disease, the confusing terminology, the pathological findings and the theories of its etiology have been discussed in several recent articles (1, 2, 3). These investigators have pointed out that widespread changes can be found in many organs of the body even though few symptoms arise from the lesions. These reports have emphasized the pathological and etiological aspects of the disease and have largely overlooked the accompanying metabolic changes. It is difficult to find recorded values for even the more common blood chemical determinations. Rarely have determinations of the non-protein nitrogen (1) and cholesterol (4) content of the blood, or attempts to find abnormal proteins in the urine, been recorded.

Attention has been called recently (5) to the hyperproteinemia found in some instances of the disease due chiefly to a rise in the globulin fraction. No report has been made previously of the variation of the serum protein content of the blood of cases observed over a period of years. No report of other abnormal blood chemical findings has been found in the literature.

The discovery of bone cysts in one of the cases which we have studied stimulated interest in this problem. These lesions were adequately described from the clinical and roentgenological standpoint by Jüngling (6) in 1928. Although exhaustive studies of calcium metabolism have been done in instances of hyperparathyroidism presenting bone cysts (osteitis fibrosa cystica), determinations of blood calcium and phosphorus in Boeck's sarcoid have been reported very rarely (1). No determinations of blood phosphatase have been recorded.

The use of the calcium tolerance test in the study of the disease has not been reported before.

It was thought that the values for normal constituents of the blood obtained over periods of months, and in some instances years, warranted recording because of the rarity of such observations, and that an attempt to correlate the variations with the lesions of the disease would be interesting. It was hoped that the results of calcium tolerance tests and the finding of abnormal proteins in the urine might prove helpful in evaluating the extent of bone lesions.

METHODS

Eleven instances of Boeck's sarcoid have been studied in Duke Hospital since 1934. Observations were repeated in Cases 1 and 5 two years after clinical recovery. The diagnosis in all cases was confirmed by biopsy of the skin or the lymph nodes.

Serum protein determinations were done by the macro-Kjeldahl method. The serum calcium concentration was determined by the method of Kramer and Tisdall (7), titrating with ceric sulfate in place of potassium permanganate (8). Blood inorganic phosphate determinations were made by Bodansky's modification (9) of Kuttner and Lichtenstein's method (10). The blood phosphatase was done by Bodansky's method (11). The bilirubin in the bilirubin tolerance test suggested by Soffer (12), was done by the method described by Malloy and Evelyn (13). The first sample was obtained at 8 minutes instead of 5 minutes. The calcium tolerance test was done as suggested by London and Bernheim (14) except for the omission of chocolate by mouth.

RESULTS

The results of the experiments are recorded in Table I. The chemical changes in Boeck's sarcoid

TABLE I

Authors' data on cases of *Borck's sarcoma*

Case	Initials	Age	Sex	Date	Duration	Active	Inactive	Site				Lymph nodes	Liver	Total proteins*	Albumin*	Globulin*	A/G ratio	Calcium Tolerance					Phosphorus †	Cholesterol †	Ketone †	Bilirubin & retention ‡	Bence-Jones	Urine		
								Bone	Skin	Hys	Lungs							Calcium	Phosphorus											
																			15 ml. †	2 hr. †	15 ml. †	2 hr. †								
1	JL	26	C	M	Dec. 13 1914 Oct. 14 1918	8 mo. 4 yrs.	+	+	+	+	+	+	+	1.3401 1.3530	6.2 8.6	2.6 3.1	1.40 1.40	31 31	8.6 9.9	3.6 3.7	3.6 3.6	4.3 4.3	240				+			
2	RP	23	C	F	June 15 1916	12 mo.	±	+	+	+	±	+	+	1.5007	7.46	2.60	4.76	0.55	27	11.1		3.8					+			
3	FM	27	C	F	Dec. 7 1916 Nov. 1 1917 Nov. 1 1918 Sept. 15 1918	7 yrs. 6 yrs. 9 yrs.	+	+	+	+	+	+	+	1.3312 1.3420 1.3420 1.3509	7.50 8.17 8.17 8.3	3.17 3.75 3.75 3.1	4.33 4.43 4.35 5.2	0.71 0.85 0.85 0.60	20 28 28 41	9.6 14.2 14.2 14.2					3.7 3.7 3.7 3.0	5.7 8.5 8.5 6.7				0
4	AB	22	Ind.	M	Mar. 17 1917 Sept. 26 1918	12 mo. 21 yrs.	0	0	+	+	+	+	+	1.3350 1.3521 1.3521 1.3492	9.29 9.37 9.1 7.0	3.59 3.51 3.51 4.4	6.00 4.76 4.76 2.6	0.35 0.57 0.57 1.70	34 31 31 22	12.8 10.2 10.2 10.8	3.6 3.6 3.6 3.2	3.0 3.2 3.2 3.2	4.9							0
5	IC	27	C	M	May 17 1917 Apr. 27 1919	2 mo. 2 yrs.	0	+	0	+	+	+	+	1.3519 1.3492	8.37 7.0	3.61 4.4	4.76 2.6	0.76 1.70	25 22	10.6 10.8	3.9 3.2				3.3	215			0	
6	JT	21	C	M	July 13 1917	16 mo.	0	+	+	+	+	+	+	1.3520	8.34	3.71	4.63	0.60	36									0		
7	MP	27	C	F	June 17 1918 Oct. 17 1918 May 10 1919	21 mo. 7 mo. 14 mo.	0	0	0	+	+	+	+	1.3483 1.3516 1.3503	7.5 8.5 8.5	3.7 3.8 3.8	1.0 1.7 1.7	11.2 11.2 11.2	3.2 3.2 3.6	4.4 16.0				4.4	161			0		
8	GB	37	W	M	Aug. 22 1918	4 mo.	±	0	0	+	+	+	+	1.3490	6.3	2.4	3.9	0.62	44	14.1		3.7	7.4	152			+			
9	BM	32	C	F	Nov. 18 1918 May 27 1919 June 3 1919	6 yrs. 6 yrs.	0	+	+	+	+	+	+	1.3506	9.3	3.4	5.9	0.58	28	10.4	11.8	11.1	3.7 4.0	3.8	17.1	133			0	
10	LB	31	C	M	April 12 1919 April 22 1919 June 3 1919	2 yrs.	±	0	+	+	+	+	+	1.3509 1.3509 1.3509	9.64 9.6 9.6	3.7	5.9	0.63	32	12.6	14.4	14.0	4.5 3.8 3.9	7.2 6.2 6.9	182 115 28.5			0		
11	JP	30	C	M	April 13 1919 April 18 1919 April 22 1919 May 22 1919 May 27 1919 June 10 1919	5 yrs.	+	+	+	+	+	+	+	1.3511 1.3510 1.3526 1.3526 1.3526 1.3510	8.5 7.5 8.5 8.5 8.5 8.5	3.0 2.1 3.2	0.54 0.40	27 27 10.6	9.7 11.1 10.6	4.1 11.1 3.0				3.1 9.7 3.1 3.0 3.1	105 168 158 105	17.4		0		

* Grams per cent. † Milligrams per cent. ‡ Bodanisky units. \$ Per cent.

* Grams per cent.

† Milligrams per cent.

‡ Bodansky units.

§ Per cent.

reported by other observers are shown in Table II. The cases of the latter group are, for the most part, of longer duration than those of the former one.

Total serum proteins above 8 grams per cent were found in 8 patients, but the reversal of the albumin-globulin ratio was present in all during the active stage. Two years after clinical recovery, normal values were found in Case 5, in whom they were previously abnormal, and in Case 1, on whom a previous determination had not been done. The highest albumin-globulin ratio observed in the active cases was 0.85, and the lowest, 0.40. Total serum proteins above 7.5 grams per cent in normal individuals are rarely observed in this laboratory.

No suggestion of the simultaneous occurrence of any of the other diseases known to cause hyperproteinemia appeared. Small amounts of a substance resembling Bence-Jones protein in its solubility at the boiling point were found in the urine of 2 cases.

The serum calcium concentration was definitely increased in 6 patients. The calcium intake was not increased in any of the patients. After the first determinations of the calcium level were made, the vitamin D intake was increased in patients 3 and 4 who took 1 pint of cod liver oil monthly. In the calcium tolerance test a rise of 1.3 to 1.5 mgm per cent in 15 minutes with return almost to the basal level in two hours was found in Cases 1, 3 and 4. In Cases 7 and 11 a flat curve with a rise of only 0.5 mgm per cent, and in Cases 9 and 10 a high plateau curve with a rise of 1.6 to 1.8 mgm per cent and a fall of only 0.4 to 0.7 mgm per cent, were noted. Serum calcium values above 10.5 mgm per cent in normal individuals are unusual in this laboratory.

The blood inorganic phosphorus levels were normal. A slight increase was noted during the calcium tolerance tests. The blood phosphatase was elevated in each case in which it was determined. The usual range was 5 to 8 Bodansky units, with the highest value, 17.1 units, in Case 9. The non-protein nitrogen of the blood was not appreciably altered.

The blood cholesterol was low or normal, 133 to 182 mgm per cent, in 6 active cases and slightly elevated, 240 mgm per cent, in another patient

who subsequently recovered. In 1 recovered case the level was 215 mgm per cent. In 3 active cases the cholesterol esters were 62, 63, and 66 per cent of the total cholesterol.

The bilirubin tolerance test showed definite retention, 62, 16, 17.4, and 28.5 per cent in the instances in which it was done.

DISCUSSION

Proteins Hyperproteinemia, with an increase chiefly in the globulin fraction, has been reported in a number of diseases (15, 16), it is found most frequently in multiple myeloma and lymphogranuloma inguinale. Similar findings in Boeck's sarcoid were first noted by Salvesen (5) and confirmed by Bing (16). Longcope and Pierson (1) did not find an increase in total serum proteins, but found an increase in globulin without reversal of the A/G ratio in 3 cases. Snapper (17) found an increase in total serum proteins with reversal of the ratio in only 1 out of 8 cases, but the globulin was elevated in 2 others. In each instance of hyperglobulinemia the disease process was active. No correlation of the level of serum proteins could be made with either the site or duration of the disease. It is suggested by Cases 6 and 7 of this series and 5 cases reported by Snapper (17) that the serum globulin and total protein concentration return to normal after the lesions heal. In lymphogranuloma inguinale the changes may persist for years (18). The increase in sedimentation rate of the red cells which has been reported in cases of hyperproteinemia and hyperglobulinemia (15) was noted. The exact mechanism of the increase in serum globulin is not known. It is possible, as suggested by Chu and Hastings (19) in the cases of lymphogranuloma inguinale studied by Gutman, Tyson, and Gutman (20), that some abnormal protein is present, but this point has not been investigated. The fractional precipitation curves of the serum proteins in Cases 3, 4, 5 were studied by Perlzweig, Kondritzer and Bruch (21), using the method of Butler and Montgomery (22), and were all found to follow the same pattern. The curves differed markedly in pattern from the normal and resembled closely those of instances of liver disease. Gutman, Tyson, and Gutman (20) have shown that the serum globulin is raised without

rise in total proteins in hepatic cirrhosis. Liver lesions have not been described in the rare autopsy sites on lymphogranuloma inguinale, but have been found in many of the autopsied instances of sarcoid. The results of the bilirubin tolerance test indicate an impaired liver function. No attempt has been made in the past to evaluate clinically the extent of liver damage in sarcoid.

Calcium. A serum calcium determination, 10.8 mgm per cent, was found in only 1 reported case (1), the bones were involved in this instance. The highest values obtained in the cases reported here were 14.8 mgm per cent in Case 8, 14.2 mgm per cent in Case 3 and 12.8 mgm per cent in Case 4. These had respectively one questionable cyst, definite bone cysts, and no demonstrable bone lesions. The next highest value, 12.6 mgm. per cent, was obtained in Case 10 which showed suspicious areas in the ribs. Case 2, with a value of 11.1 mgm per cent, showed only rarefaction. Case 11, which had definite cysts in the toes and trabeculation in the fingers, had a level of 10.6 mgm per cent. In the other cases no lesions were demonstrable in x ray films of hands (all cases), feet (8 cases) long bones (3 cases), ribs (all cases), lumbar spine and pelvis (2 cases), and skull (2 cases).

These values are definitely higher than can be accounted for by the hyperproteinemia, for Gutman, Tyson, and Gutman (20) have reported that the amount of calcium bound to globulin is fairly constant. From the determination of ionization constants of calcium in concentrated normal sera Chu and Hastings (19) believe that some abnormal protein is present in the cases studied by Gutman, Tyson, and Gutman, and thus the proteins fail to combine with the expected amount of calcium. This may be true, but the calcium level in this series does not vary in proportion to either the total protein albumin or globulin content. The changes in blood phosphorus or phosphatase show no relation to the calcium level. In multiple myeloma (20) the high serum calcium in the presence of hyperproteinemia is believed to be accounted for by the increased bone destruction. In Case 3, an increase in the serum calcium over a period of two years was associated with slight increase in the lesions of the feet. Schaumann points out (2) that the degree of bone marrow involvement in sarcoid cannot be judged by the

x ray findings, for foci can be found by pathological examination of the tissue when none appeared in x ray films. It is thought that the blood calcium may indicate this activity but studies of a greater number of cases over a period of years are needed to settle this point. Elevation of the serum calcium has not been found in cases of tuberculosis, which most closely resemble sarcoid pathologically or in other destructive infectious diseases of bone (23).

The possible relationship of the parathyroid glands to these findings is of interest. In Case 3 a nodule was palpable at the upper pole of the left lobe of the thyroid on the last examination, this case had definite bone cysts. No attempt was made to assay the parathormone content of blood in the patients reported here. It was thought that the calcium tolerance test as used by London and Bernheim (14) offered a simple method of gauging the utilization of calcium. In Paget's disease the calcium curves are flat, presumably because the osteoid tissue rapidly removes calcium from the blood stream. If the parathormone level were increased, the curve would be expected to rise and to remain elevated. We have had no opportunity to check this with cases of hyperparathyroidism. Hyperplasia of the parathyroids has not been reported in sarcoid. In Case 11, with definite bone cysts, and in Case 7 with no bone lesions flat calcium curves, as in Paget's disease were obtained. In Case 3 with bone cysts which were increasing in size, a normal curve was obtained, as in Cases 1 and 4 with no bone lesions. Case 10, with suspicious areas in the ribs and Case 9, with no bone lesions, had plateau curves. The significance of these findings is not clear. Calcification in the kidney parenchyma or renal stones, which are often seen in hyperparathyroidism were not encountered in these cases.

Phosphorus. The normal blood phosphorus levels are further evidence that no hyperfunction of the parathyroid glands was present (20). The phosphorus values were not elevated by renal retention, since no impairment of kidney function was apparent from repeated urine examinations or the blood non protein nitrogen levels.

Phosphatase. The changes in the blood phosphatase level in diseases of bone have been discussed recently by other investigators (20, 23).

TABLE II

Data on cases of Boeck's sarcoid from literature

	Author	Case number	Age	Color	Sex	Date	Duration	Active	Inactive	Site						Blood								Urine					
										Bone	Skin	Eye	Lungs	Lymph nodes	Liver	Total proteins*	Albumin*	Globulin*	A/G ratio	Fibrinogen*	N P N †	Calcium †	Phosphorus †		Cholesterol				
1	Goeckerman (6)	5	54		M	1928	1-10 yrs	+			++		++				9.00	3.02	5.98	0.51									Bence-Jones
2	Salvesen (4)	1	53		F	Apr 16, 1935	6 yrs	+		0	+++	0	+++	0	+	9.21	3.11	6.10	0.51										
3		46	M	May 18, 1935	9 yrs	+		++	++	++	++	++	++	++	0	9.40	4.35	5.05	0.86	0.92									
4		38	F	Feb 2, 1935	9 yrs	+		++	++	++	++	++	++	++	++	0	9.69	3.85	5.84	0.66									
						Apr 2, 1935										9.17	3.35	5.82	0.57										
5	Longcope & Pierson (1)	4	21	C	F	Feb 13, 1929	4 yrs	+		+++	++	++	++	0														0	
6		2	24	C	M	Jan 15, 1936	11 yrs	?		++	Healed	0	++	+	+	7.32	4.31	3.0	1.43			34							
7		6	28	C	M	Jan 30, 1936	1 yr	+		+	±	++	++	++	++	6.79	3.53	3.25	1.08			26							
8		7	43	C	F	Jan 30, 1936	5 yrs	+		+++	0	±	++	++	++	6.59	3.49	3.09	1.13			36							
9	Bing (16)	22				1937										8.61	3.01	5.60	0.54										
10		24														7.91	0.97	6.94	0.14										
11	Jeghers & Selesnick (15)	8	27		F	1937										8.4													
12	Wise & Gutman (25)	39				1937										8.5	4.0	4.5	0.88										
13	Snapper (17)	5	18		M	1936	1 yr	++		++	++	++	++	++	++	8.85	3.97	4.52	0.88	0.36									
14		8	15		F		6 yrs	++		++	++	++	++	++	++	8.28	4.52	3.27	1.38	0.47									
15		11	21			1935		+		+	++	++	++	++	++	7.81	3.79	3.60	1.06	0.42									
16		1	12		F			?		0	++	++	++	++	++	6.22	3.65	2.08	1.76	0.48									
17		10	17		F		5 yrs			++	++	++	++	++	++	7.12	4.91	2.21	2.22										
18		13	16		F		4 yrs			++	++	++	++	++	++	7.51	4.80	2.25	2.13	0.26									
19		3	15		M		2 1/2 yrs			0	0	Healed	++	++	++	7.59	5.08	2.25	2.26	0.26									
20		4	15		M							++	++	++	++	7.47	4.54	2.61	1.74	0.32									
21	Scott (26)	8	55		F	1938	13 yrs	+		++	+	0	++	±		10.4	5.2	5.2	1.00										

* Grams per cent.

† Milligrams per cent.

‡ Increased, Split 55

12. Soffer L. J., Present day status of liver function tests. *Medicine*, 1935 14 185
13. Malloy, H. T., and Evelyn, K. A., Determination of bilirubin with photoelectric colorimeter *J Biol. Chem.*, 1937 119 481
14. London, I. M., and Bernheim, A. R., Calcium tolerance curves in Paget's disease of bone. *J Lab. & Clin. Med.*, 1937 23 18.
15. Jeghers, H., and Selesnick, S., Hyperproteinemia its significance. *Internat. Clin.* 1937, 3 248.
16. Bing J., The formolgel reaction and other globulin reactions. *Acta Med. Scandinav.*, 1937 91 336.
17. Snapper I., Pseudo-tuberculosis in man. *Haarlem Bohn*, 1938.
18. Gutman A. B., and Gutman, E. B., Relation of serum calcium to serum albumin and globulins. *J Clin. Invest.*, 1937 16 903
19. Chu, H. I. and Hastings, A. B., Note on state of calcium in high protein serum. *J Clin. Invest.*, 1938, 17, 167
20. Gutman, A. B., Tyson, T. L., and Gutman, E. B., Serum calcium, inorganic phosphorus and phosphatase activity in hyperparathyroidism, Paget's disease, multiple myeloma and neoplastic disease of bones *Arch. Int. Med.* 1936 57 379
21. Perlzweig W. A., Kondritzer A. A., and Bruch, E., Preliminary report The solubility precipitation patterns of the serum proteins. *Proc. Amer. Soc. Biol. Chem.*, 1938, XCII (Complete report to be published)
22. Butler A. M., and Montgomery H., The solubility of plasma proteins dependence on salt and plasma concentrations in concentrated solutions of potassium phosphate. *J Biol. Chem.*, 1932, 99 173
23. Mitchell C. L., and Crawford, R. R., Serum phosphatase—its clinical application in diseases of bone. *J Bone & Joint Surg.*, 1937 19 630.
24. Bodansky, A., and Jaffe, H. L., Phosphatase studies serum phosphatase in diseases of bone interpretation and significance. *Arch. Int. Med.*, 1934 54, 88.
25. Wise, C. R., and Gutman, A. B., Formol-gel reaction convenient preliminary test for hyperglobulinemia. *Am. J. M. Sc.*, 1937 194 263
26. Scott, R. B., Sarcoidosis of Boeck. *Brit. M. J.*, 1938, 2, 777

24) It is generally agreed that an increase is indicative of new or abnormal bone formation, or of decalcification, even though the serum calcium level is normal. In infections of bone, pyogenic or tuberculous, or in fractures, the phosphatase level is little changed (23). The difficulty in determining the extent of bone lesions in sarcoid has been pointed out. The phosphatase content of the blood could not be correlated with the extent or activity of bone lesions as demonstrated by x-ray, or with the calcium level. In Case 3, with definite bone cysts, a progression of the lesions in the bones of the feet was not accompanied by a progressive rise in phosphatase. An elevated calcium and phosphatase with a normal phosphorus level are found occasionally in multiple myeloma and metastatic malignant bone lesions. Phosphatase is known to be present in many tissues. With a high blood level, in the absence of bone lesions, disturbances of the liver are suggested (24). The liver was enlarged in Cases 1, 3 and 8, but ascites or jaundice was not present in any. The excretory function of the liver was impaired in the 4 patients tested. No correlation could be deduced from the involvement of other organs or the duration of the disease. It is suggested that the elevation of the blood phosphatase indicates activity of lesions in various organs and not in the bones and liver alone.

Cholesterol In lymphogranuloma inguinale (18), the blood lipids have been found to be decreased, with the trend less marked in the cases of short duration. The value of this determination in cases of sarcoid must await further study.

Bence-Jones Protein In no cases in which material resembling Bence-Jones protein was present in the urine were definite bone lesions demonstrated by x-ray, one instance showed decalcification of the bones of the hands and the other a questionable cyst in the fingers. None was demonstrated in the urine of the cases with definite bone involvement.

SUMMARY

The blood chemical changes found in 11 cases of generalized Boeck's sarcoid studied over a period of years are reported. The occurrence of hyperproteinemia and hyperglobulinemia in active cases is confirmed, and evidence that these ab-

normalities disappear as the lesions heal is presented.

The serum calcium is elevated in many cases. Calcium tolerance tests give variously shaped curves. The blood phosphatase is elevated in all active cases. The blood phosphorus level is unchanged.

The blood chemical data indicate that the changes in the bones are not associated with hyperfunction of the parathyroid glands.

The occasional occurrence in the absence of demonstrable bone lesions of substances in the urine resembling Bence-Jones protein is reported.

Hepatic function is impaired as shown by abnormal bilirubin excretion, fractional precipitation patterns of the serum proteins, and an increase in blood phosphatase.

BIBLIOGRAPHY

- 1 Longcope, W. T., and Pierson, J. W., Boeck's sarcoid (sarcoidosis). *Bull. Johns Hopkins Hosp.*, 1937, 60, 223.
- 2 Schaumann, J., Lymphogranulomatosis benigna in light of prolonged clinical observations and autopsy findings. *Brit. J. Dermat.*, 1936, 48, 399.
- 3 Pinner, M., Noncaseating tuberculosis, analysis of literature. *Am. Rev. Tuberc.*, 1938, 37, 690.
- 4 Goeckerman, W. H., Sarcoids and related lesions. Report of seventeen cases, review of recent literature. *Arch. Dermat. and Syph.*, 1928, 18, 237.
- 5 Salvesen, H. A., The sarcoid of Boeck, disease of importance to internal medicine. *Acta Med. Scandinav.*, 1935, 86, 127.
- 6 Jüngling, O., Über Ostitis tuberculosa multiplex cystoides, zugleich ein Beitrag zur Lehre von dem Tuberkuliden des Knochens. *B. Beitr. z. Klin. Chir.*, 1928, 143, 401.
- 7 Kramer, B., and Tisdall, F. F., A simple technique for determination of calcium and magnesium in small amounts of serum. *J. Biol. Chem.*, 1921, 47, 475.
- 8 Larsen, C. E., and Greenberg, D. M., Analysis of calcium in blood and other biological material by titration with ceric sulfate. *J. Biol. Chem.*, 1938, 123, 199.
- 9 Bodansky, A., Phosphatase studies. Determination of inorganic phosphate. Beer's law and interfering substances in the Kuttner-Lichtenstein method. *J. Biol. Chem.*, 1932, 99, 197.
- 10 Kuttner, T., and Lichtenstein, L., Micro colorimetric studies. Estimation of phosphorus molybdic acid—stannous chloride reagent. *J. Biol. Chem.*, 1930, 86, 671.
- 11 Bodansky, A., Phosphatase studies, determination of serum phosphatase. Factors influencing the accuracy of determination. *J. Biol. Chem.*, 1933, 101, 93.

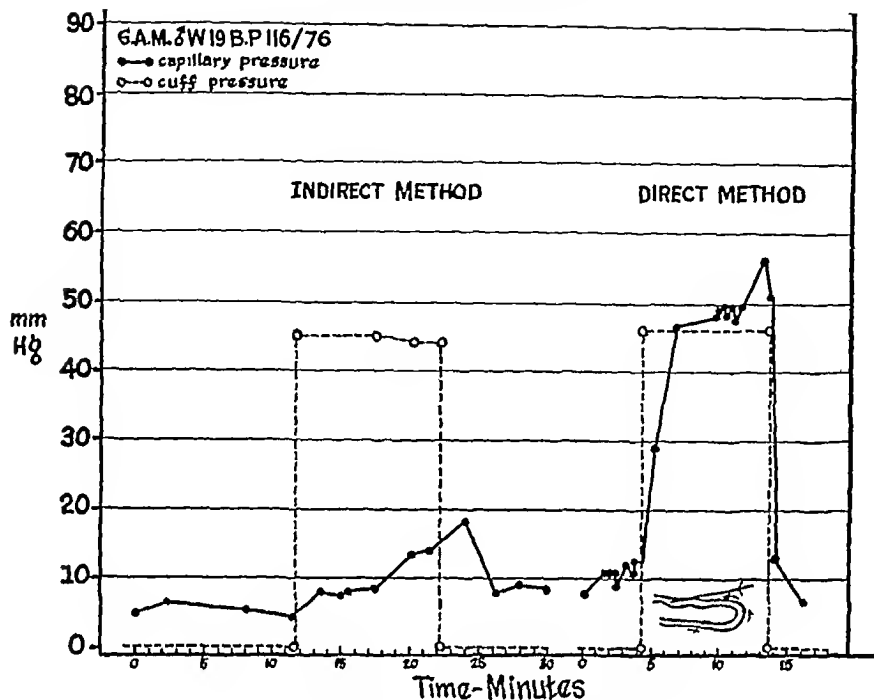


FIG. 1 RESPONSE OF CAPILLARY BLOOD PRESSURE TO INCREASED VENOUS PRESSURE IN A SUBJECT WITH NORMAL BLOOD PRESSURE

Indirect method no significant rise. Direct method (venous limb) prompt rise to exceed cuff pressure.

All charts are similarly plotted. The ordinates represent millimeters of mercury for both cuff and capillary pressures the abscissae, time in minutes. The solid line indicates the observed capillary pressure reading by both the direct and indirect methods. The dotted line is cuff pressure. In the diagram of the capillary the straight line represents the location of the pipette in the capillary loop the arrows, the direction of capillary blood flow. The first line of the key in the upper left hand corner of the charts indicates, in order the subject's initials sex color age, and blood pressure. The method which was employed first is charted first.

variably records a prompt rise in capillary blood pressure, the elevation persisting throughout the entire period that the cuff is inflated.

Observations by the direct method were made upon 31 different capillaries in 18 subjects 9 with normal 7 with high and 2 with low arterial blood pressure. In each of these capillaries a prompt rise in blood pressure was always recorded during the critical test. In 21 capillaries the pressure rose to exceed the cuff pressure. In the remain-

ing 10 (to be discussed subsequently) the capillary pressure rose promptly but failed by 2 to 5 mm Hg to reach the level of the cuff pressure. In well over 200 capillaries studied by the indirect method the capillary pressure readings during the critical test showed either no change, or at the most only slight and inconstant rises above the initial level. In 9 experiments a single capillary was studied by each of the two methods during the critical test always with the above mentioned

a manometer system in which the pressure may be changed at will by means of a plunger driven by a fine thumb-screw. The micropipette, brass tube and manometer system are filled with Ringer's solution (pH 7.3 to 7.4), with heparin (3 mgm per 100 cc. of solution) added as an anticoagulant.

In determining the capillary blood pressure, the tip of the micropipette is carefully introduced into the lumen of a capillary loop at the desired location. Since the initial pressure of the solution in the micropipette is only 2 to 3 mm. Hg, the entry of the micropipette into the capillary is signaled by a rush of blood into the pipette tip. As soon as this occurs, the pressure in the micropipette is quickly raised, forcing most of the red blood cells back into the capillary. By further adjustment of the pressure, a state of equilibrium is reached in which a few red blood cells remain in the extreme tip of the micropipette, oscillating back and forth with each heart beat, but manifesting no tendency to move progressively either into or out of the tip. The manometer reading at this equilibrium point, after corrections have been made for the capillarity of the manometer and for the difference in level between the base of the manometer and the level of the nail fold, represents the capillary blood pressure. The balance between manometer pressure and capillary pressure at the equilibrium point is a very delicate one; changes of 2 to 3 mm Hg in manometer pressure suffice to establish progressive movement of the red cells either into or out of the micropipette. Throughout this entire procedure the capillary blood flow should not be varied from the normal, swiftly-flowing stream. If the blood flow appears to be obstructed or otherwise altered, pressure readings are considered unacceptable. It has been necessary to discard a number of readings on this account.

In order to facilitate the passage of the fragile micropipette through the skin, the most superficial layers of the epidermis of the nail fold were always cut away with a keen razor blade. Only non-living tissue was removed, and pain, bleeding and tissue damage were avoided. This procedure was carried out approximately an hour before determinations of pressure were made. The nail folds were prepared in this manner before both the direct and indirect measurements.

The direct method has several shortcomings. (1) The technical difficulties are formidable. (2) The insertion of the micropipette frequently causes such changes in capillary blood flow (stasis) that acceptable pressure readings cannot be obtained. (3) Determinations of pressure can be made on only one capillary at a time, hence, relatively few capillaries can be studied in the course of a single experiment.

The critical test

In order to evaluate the reliability of the two foregoing methods, both have been subjected to what may be called a critical test. This test is carried out in the following manner. A pneumatic

cuff (width 14 cm) is placed loosely about the arm above the elbow. After several determinations of capillary pressure have been made, the venous pressure distal to the cuff is raised by inflating the cuff to a pressure below diastolic arterial pressure. During the ensuing period of elevated venous pressure, the determinations of capillary pressure are repeated. The cuff is then deflated, allowing the venous pressure to fall to its original level, and the capillary pressure determinations are again repeated.

The following considerations led to the use of this type of critical test. When a pneumatic cuff encircling the upper arm is inflated, the venous pressure distal to the cuff rises and within one minute very closely approximates the pressure within the cuff (Lewis (7)), provided the cuff-pressure does not exceed diastolic arterial pressure. Under these circumstances, in spite of the increased venous pressure, the blood flow in the capillaries of the nail fold can be seen to persist steadily onward, though usually at a slower rate. This persistence of capillary blood flow indicates that the capillary blood pressure has risen to exceed the elevated venous pressure. Landis (5), in testing the accuracy of the direct method, made use of this principle, and observed that the directly-determined capillary pressure rose to exceed the cuff-pressure. It is believed, therefore, that a reliable method for the determination of capillary blood pressure should show that capillary pressure exceeds venous pressure so long as blood is flowing through the capillaries into the veins.

RESULTS

Relation of capillary pressure to occluding cuff-pressure

The charts indicate the type of results obtained when each of the two methods was subjected to the critical test. The capillaries were studied by each of the methods during the same observation period and hence under relatively uniform conditions. The method which was employed first is charted first.

Figures 1 to 4 exemplify the difference in results obtained by the two methods. The indirect method fails to detect any significant change in capillary blood pressure when the venous pressure rises. On the other hand, the direct method in-

by 2 to 5 mm. Hg to reach cuff pressure (Figure 5) In checking over the set up of the experiments, it was noted that the nail fold had usually been at a level materially higher than that of the most dependent portion of the occluding cuff. The question arose whether this difference in level between the hand and the cuff might lead to a discrepancy between the pressure in the veins of the hand and that in the cuff. To answer this question the pressure was determined in the veins of the dorsum of the hand* at various occluding cuff pressures, while the relation of the level of the hand to the level of the cuff was being changed through a wide range. Table I gives the results

TABLE I
Relation of venous pressure in the hand to cuff pressure during change of level of hand

Relation of hand vein to most dependent part of cuff	Cuff pressure	Venous pressure	Difference
	mm. Hg	mm. Hg*	mm. Hg
13.0 cm. above	60	54.3	-5.7
9.7 cm. above	60	56.1	-3.9
5.4 cm. above	60	57.9	-2.1
1.6 cm. above	60	61.3	+1.3
1.7 cm. above	60	62.5	+2.5
1.7 cm. above	52	54.3	+2.3
1.7 cm. above	34	35.5	+1.5
3.5 cm. below	34	42.1	+8.1
3.5 cm. below	52	59.2	+7.2

* Actually determined in mm. of isotonic salt solution and converted to mm. Hg

* By the direct method of Moritz and v. Tabora.

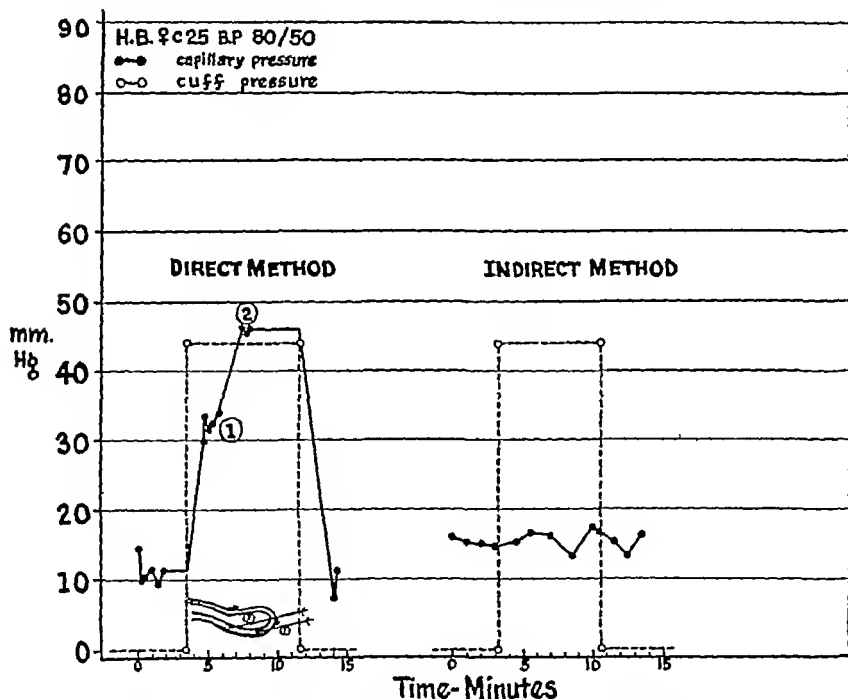


FIG 3 RESPONSE OF CAPILLARY BLOOD PRESSURE TO INCREASED VENOUS PRESSURE IN A SUBJECT WITH ARTERIAL HYPOTENSION

Direct method (venous limb) capillary pressure finally exceeded cuff pressure. During readings at (1) flow was obstructed by the pipette. Indirect method capillary pressure unaltered.

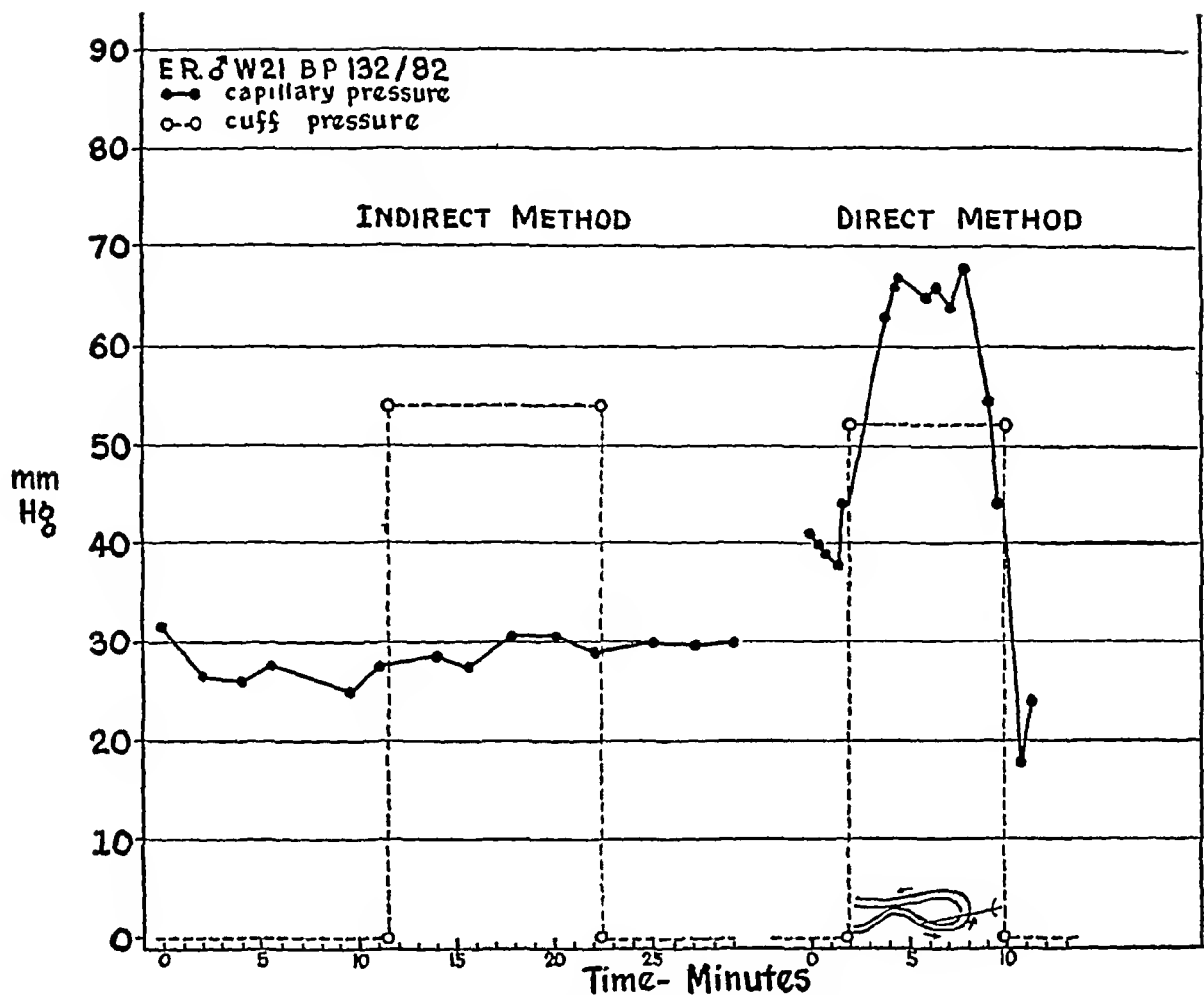


FIG. 2 RESPONSE OF CAPILLARY BLOOD PRESSURE TO INCREASED VENOUS PRESSURE IN A SUBJECT WITH NORMAL BLOOD PRESSURE

Indirect method no change whatever Direct method (arteriolar limb) quick response to exceed cuff pressure. Capillary stasis at the time of the last four readings probably accounts for their low level.

difference in result The capillary pressure response typical for each method was obtained at all cuff-pressures between the limits of 36 and 90 mm Hg The higher cuff-pressures were employed in hypertensive subjects, in one of whom a direct capillary pressure reading of 102 mm Hg was obtained at a cuff-pressure of 90 mm Hg

Since the capillary blood flow persisted steadily onward during the period of elevated venous pressure in all of the foregoing experiments, it may be assumed that the capillary blood pressure actually exceeded the venous pressure Therefore, it has been concluded, that, whereas the direct method yields accurate estimates of capillary blood pressure, the indirect method fails to do so

The direct method has also shown that rapid fluctuations in capillary blood pressure may occur spontaneously (see Figures 4 and 6A) These are not detected by the indirect method

Relation of venous pressure in the hand to occluding cuff-pressure

In the earlier experiments with the critical test it was assumed that the pressure in the occluding cuff accurately represented the pressure in the veins of the hand It became necessary to inquire whether this was a correct assumption when observations (see above) were recorded in which the directly-determined capillary pressure, although rising promptly with cuff inflation, failed

erroneous conclusions may also be reached in interpreting those observations in which, during the critical test the capillary pressure exceeded the cuff pressure. Figure 6B is an example of this. It shows that, whereas the capillary pressure was higher than cuff pressure by 0.5 to 8 mm Hg the measured venous pressure was exceeded by 4 to 13 mm Hg.

Subsidiary experiments with the indirect pressure capsule method

The possibility was considered that the apparent inadequacy of the indirect method to measure changes in capillary pressure might be attributed

to some local peculiarity of the nail fold capillaries. Consequently, attempts were made to apply the pressure-capsule to other skin areas in which venous pressure could be altered as required for the critical test. Most of the skin areas of the extremities were found to be either too uneven or too yielding to permit proper application and use of the capsule. The skin overlying the flat antero-medial surface of the tibia afforded an excellent site, but here the capillary pressure readings were vitiated by the spontaneous intermissions in capillary blood flow which are so frequent in this area (8).

Changing the position of the nail fold in rela-

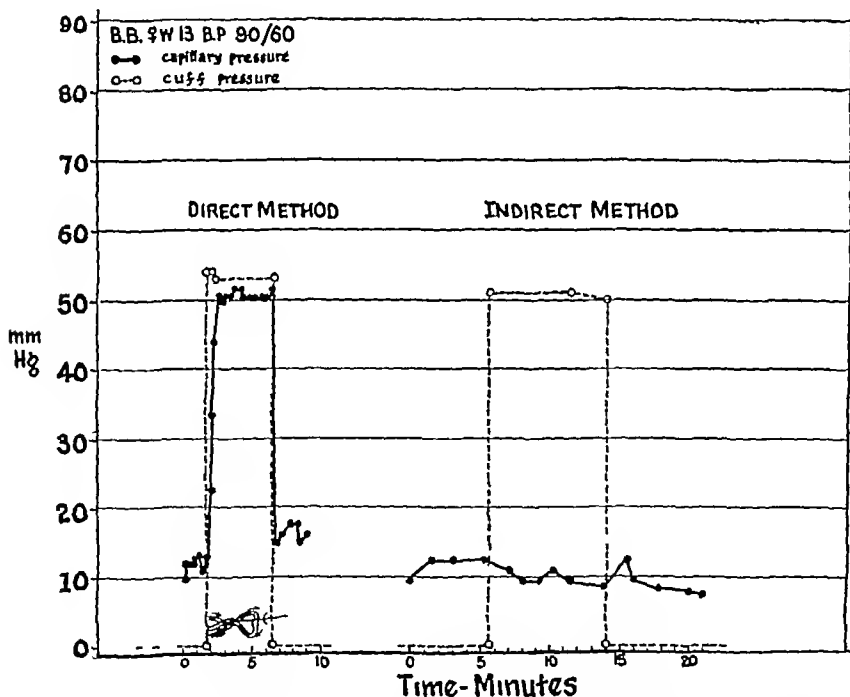


FIG. 5 RESPONSE OF CAPILLARY BLOOD PRESSURE TO INCREASED VENOUS PRESSURE IN A DIABETIC WITH NORMAL BLOOD PRESSURE

Direct method (venous limb) capillary pressure rises and falls promptly but fails to reach cuff pressure by 1.5 to 3.0 mm. Hg. Indirect method unchanged capillary pressure.

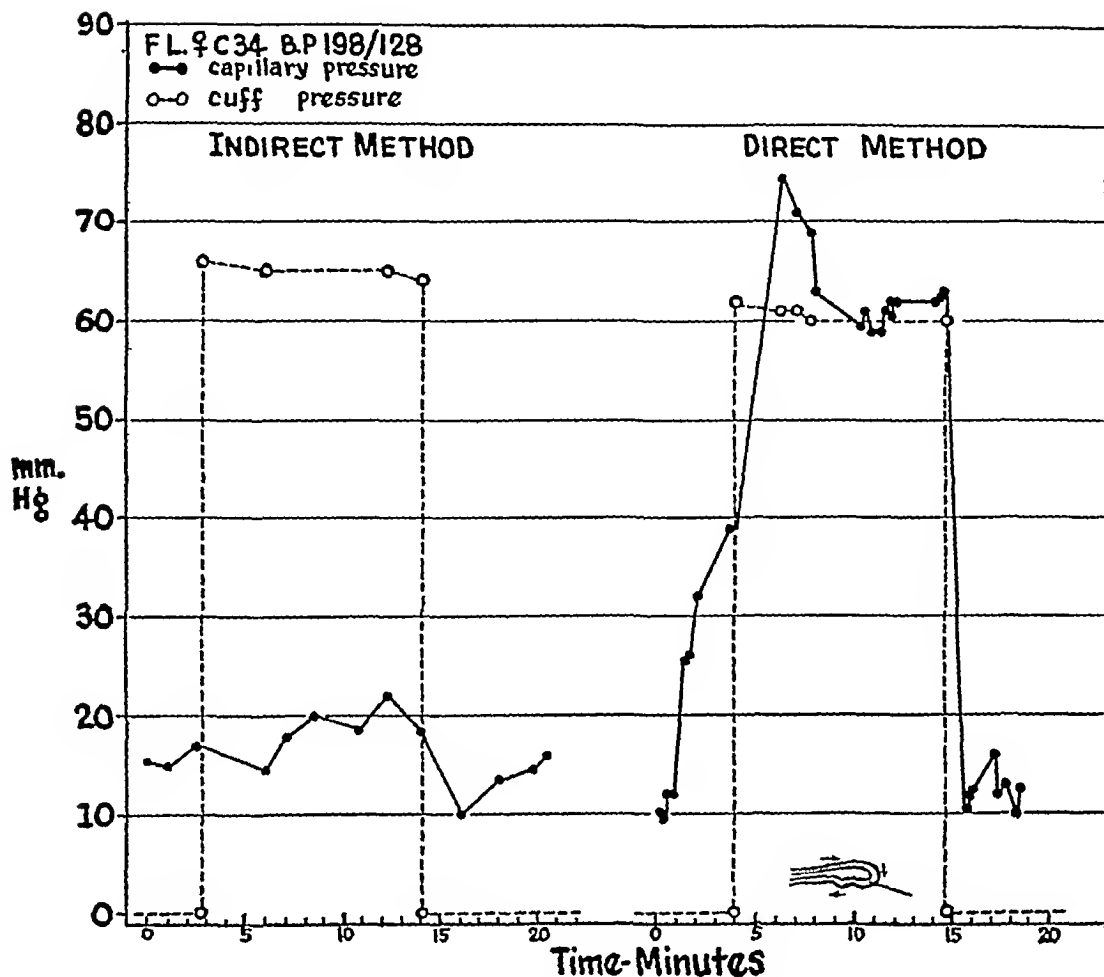


FIG 4 RESPONSE OF CAPILLARY BLOOD PRESSURE TO INCREASED VENOUS PRESSURE IN A SUBJECT WITH ARTERIAL HYPERTENSION

Indirect method no significant change Direct method (summit of loop) prompt response to exceed cuff pressure. Note spontaneous rise in capillary pressure in the period before cuff inflation.

obtained in a typical experiment. Similar results were obtained in six additional experiments.

These experiments show that venous pressure is less than cuff-pressure when the hand vein is at a level above that of the most dependent portion of the cuff. The difference between the two pressures can be accounted for by the hydrostatic relation between the level of the hand and that of the cuff.

During the critical test the nail fold was usually 9 to 12 cm above the level of the most dependent portion of the occluding cuff. This indicates that the venous pressure in the hand was usually 3 to 6 mm Hg less than cuff-pressure. Direct venous

pressure measurements in several experiments have confirmed this occlusion.

Having established these relationships, it was concluded that capillary pressure during the critical test had probably exceeded the venous pressure in the hand in all experiments, even in those in which the cuff-pressure had not been reached. Figure 6A gives evidence of the correctness of this conclusion. In this observation the directly-determined capillary pressure failed by 1.5 to 4.5 mm Hg to reach cuff-pressure but exceeded the actually measured venous pressure (horizontal arrow) by 6 to 1.5 mm Hg.

Unless these relationships are borne in mind,

THE VITAMIN C REQUIREMENT OF MAN¹ ESTIMATED AFTER PROLONGED STUDIES OF THE PLASMA CONCENTRATION AND DAILY EXCRETION OF VITAMIN C IN 3 ADULTS ON CONTROLLED DIETS

By ELAINE P. RALLI, GERALD J. FRIEDMAN AND SOL SHERRY

(From the Department of Medicine New York University College of Medicine and the Third
(New York University) Medical Division Bellevue Hospital New York City)

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There has been a wide variation in the daily estimated vitamin C requirement of man (1). This appears to be due to several factors. The first of these is the lack of uniformity in the criteria used for estimating the amount of vitamin C required; some observers using changes in capillary fragility, others using the urinary excretion following test doses of vitamin C, and still others using the amount of vitamin C excreted in 24-hour periods. Secondly, in a great many of the reports the diets are not carefully controlled. Thirdly, no prolonged studies have been done in which the plasma concentration and the urinary excretion of vitamin C have been correlated when the patients were kept on vitamin C free diets and fed quantitative amounts of vitamin C.

The question of the requirement of vitamin C can be considered from two aspects: (1) the amount necessary to maintain the normal plasma concentration, and (2) the amount necessary to raise the plasma concentration from a subnormal to a normal level. The consensus of opinion at the present time is that the normal plasma concentration of vitamin C should not be below 0.8 mgm per cent. (2). In most groups of normals reported, including a group of students observed in this laboratory, the average plasma vitamin C was about 1.2 mgm per cent.

In a previous study (3) we found that the amount of vitamin C excreted in the urine depended on the plasma concentration of the vitamin, on the rate of glomerular filtration, and on the maximal rate of tubular reabsorption. These two latter factors were found to be fairly constant in normal adults, and a sharp increased excretion of the vitamin did not occur until the plasma level had exceeded about 1.4 mgm per cent. On the basis of these observations it seemed to us that

the amount of vitamin C required daily by an adult would be the smallest amount necessary to maintain the normal plasma level. It follows that at this level the amount excreted should be small and should remain fairly constant so that the maximal reabsorptive capacity of the kidney tubules would not be exceeded. Furthermore, if more than the daily requirement of vitamin C were given, the excretion of vitamin C should rise promptly.

Obviously, in order to determine the amount of vitamin C required daily by adults, the dietary intake should be controlled, the vitamin should be fed quantitatively, the 24-hour urinary excretion of the vitamin should be determined daily, and this should be correlated with the plasma concentration at frequent intervals.

METHODS

Three normal adults were hospitalized and fed diets containing not more than 5 mgm. of vitamin C (Table I). The vitamin C content of these diets was determined by direct analysis except for the meat, the figure for which was obtained from the publication of the Department of Agriculture No. 275 (4). The 24-hour urinary excretion of vitamin C was determined daily in each case during the entire period of observation. The urines were collected in dark bottles to which was added enough glacial acetic acid so that, by the end of the 24 hours, the concentration of acid was approximately 10 per cent by volume. As the urinary volume was fairly constant in each individual from day to day, the amount of acid necessary was readily calculated. The bottles were kept in the icebox and the urines were added when voided. The plasma concentration was determined three times weekly throughout the study.

In the early part of the study, vitamin C was determined in both blood and urine by titration with dichlorophenolindophenol. Later the determinations of vitamin C were done in blood and urine in the photoelectric colorimeter using the methods described by Mindlin and Butler (5) for blood plasma and by Evelyn (6) for urine. In 2 patients on the vitamin C free diet, the urinary vitamin C was

¹This research was aided by a grant from the Josiah Macy Jr. Foundation.

obtain has been included in this report. The authors are also indebted to Mr William A Oktavec, Jr, for his constant and very helpful assistance.

BIBLIOGRAPHY

- 1 Boas, E. P., and Frant, S., Capillary blood pressure in arterial hypertension. *Arch Int. Med*, 1922, 30, 40
- 2 Ellis, L. B., and Weiss, S., Measurement of capillary pressure under natural conditions and after arteriolar dilatation in normal subjects and in patients with arterial hypertension and with arteriosclerosis. *J Clin. Invest.*, 1930, 8, 47
- 3 Mufson, I., Study of capillary pressure in nephritis and hypertension. *Am J Med Sc.*, 1932, 183, 632
- 4 Landis, E. M., Capillary pressure and capillary permeability. *Physiol Rev*, 1934, 14, 404
- 5 Landis, E. M., Microinjection studies of capillary blood pressure in human skin. *Heart*, 1930, 15, 209
- 6 Danzer, C. S., and Hooker, D. R., Determination of capillary blood pressure in man with the micro-capillary tonometer. *Amer Jour Physiol*, 1920, 52, 136
- 7 Lewis, T., Force exerted by minute vessels of the skin in contracting. *Heart*, 1924, 11, 109
- 8 Bordley, J., III, Grow, M. H., and Sherman, W. B., Intermittent blood flow in capillaries of human skin. *Bull Johns Hopkins Hosp*, 1938, 62, 1
- 9 Lewis, T., and Landis, E. M., Observations upon the vascular mechanism in acrocyanosis. *Heart*, 1930, 15, 229
- 10 Landis, E. M., Micro-injection studies of capillary blood pressure in Raynaud's disease. *Heart*, 1930, 15, 247

centration was 0.30 mgm per cent. For the first 6 days the patient was maintained on diet alone. The daily excretion was 64 with a Standard Deviation of ± 3.9 . The patient was then fed 100 mgm of cevitamic acid daily for a period of 52 days. The average daily urinary excretion for this period was 60 ± 4.4 mgm. The retention averaged 94 mgm daily. After 25 days the plasma concentration was 0.77 mgm per cent. Nineteen days later the plasma concentration was 1.07 mgm per cent, and it remained at approximately this level. The studies were then discontinued for a period of 2 months. In the second part of the study, during the first period of 8 days on diet alone, the patient excreted an average of 6 mgm with a Standard Deviation of ± 2.0 daily and the plasma concentration of vitamin C fell from 0.56 to 0.27 mgm. per cent. For the next 41 days he received 50 mgm daily. The average excretion during this period was 9.6 ± 5.2 mgm, and the average retention was 40 mgm. The average plasma concentration of vitamin C was 0.28 mgm per cent and the blood plasma varied from 0.24 mgm per cent to 0.34 mgm per cent. In the third period consisting of 43 days, the daily intake of vitamin C was increased to 75 mgm of cevitamic acid. The daily excretion averaged 7.9 ± 3.6 mgm so that the daily retention was 67 mgm. The blood plasma concentration of vitamin C rose from 0.30 mgm per cent to 0.70 mgm per cent and was maintained at this latter figure for a period of 20 days. The vitamin C intake was then increased to 100 mgm daily for a period of 45 days. The average daily excretion was 13 ± 3.7 mgm and the daily retention averaged 87 mgm. The plasma concentration of the vitamin rose from 0.70 mgm per cent to 1.20 mgm per cent within 16 days and was maintained at this level for 24 days.

During the next period of 31 days the patient received 150 mgm of cevitamic acid daily. The average daily excretion was $68 \text{ mgm.} \pm 14$ mgm and the daily retention averaged 82 mgm. The blood plasma concentration of vitamin C promptly rose from 1.20 mgm per cent to 1.50 mgm per cent and was maintained at the higher level for the rest of the period.

For the sixth and last period of 30 days the vitamin C intake was reduced to 50 mgm daily.

The purpose of decreasing the intake for a period of time was to find out whether once the plasma concentration had been elevated a smaller daily dose would maintain the higher plasma level. As can be seen in the chart this did not occur. On the 15th day the plasma concentration fell to 0.76 mgm per cent and leveled off at 0.6 mgm per cent. The urinary excretion fell more promptly than the blood and by the 7th day was 13 mgm which was the average daily excretion for the period with a Standard Deviation of ± 4.3 mgm.

A third patient A. T., 35 years of age, height 65 inches, weight 135 pounds surface area 1.65 square meters (Figure 3) was kept on the diet alone for a period of 12 days. His blood plasma concentration of vitamin C at the start of the study was 1.7 mgm per cent due to the fact that he had received massive doses of cevitamic acid for several days prior to the study. On diet alone the blood plasma concentration of the vitamin fell rapidly to 0.30 mgm per cent. The urinary excretion fell during this time and by the 11th day he was excreting none.

In the second period of 18 days he was given 50 mgm. of cevitamic acid daily. The plasma concentration of vitamin C was maintained throughout this period at a level of about 0.30 mgm per cent. The average daily excretion of vitamin C was 5.9 ± 2.3 mgm and the average daily retention was 44 mgm.

The daily intake of vitamin C was then raised to 75 mgm for a period of 25 days. The blood plasma concentration of vitamin C rose slightly to 0.50 mgm per cent. The average daily excretion was 6 ± 2.2 mgm. and the average daily retention was 69 mgm.

In the fourth period of 42 days the patient was given 100 mgm of cevitamic acid daily. The daily urinary excretion averaged 12 ± 3.3 mgm and the average daily retention was 88 mgm. The blood plasma concentration began to rise rapidly from 0.50 mgm. per cent and after 32 days had reached a concentration of 1.0 mgm per cent. For the last 10 days of the period the average plasma level was 1.2 mgm. per cent. During this fourth period the plasma concentration fell on the 25th and 27th days and we found that through an error only 50 mgm of cevitamic acid was given for 2 days prior to this fall.

In the fifth period of 18 days the vitamin C in-

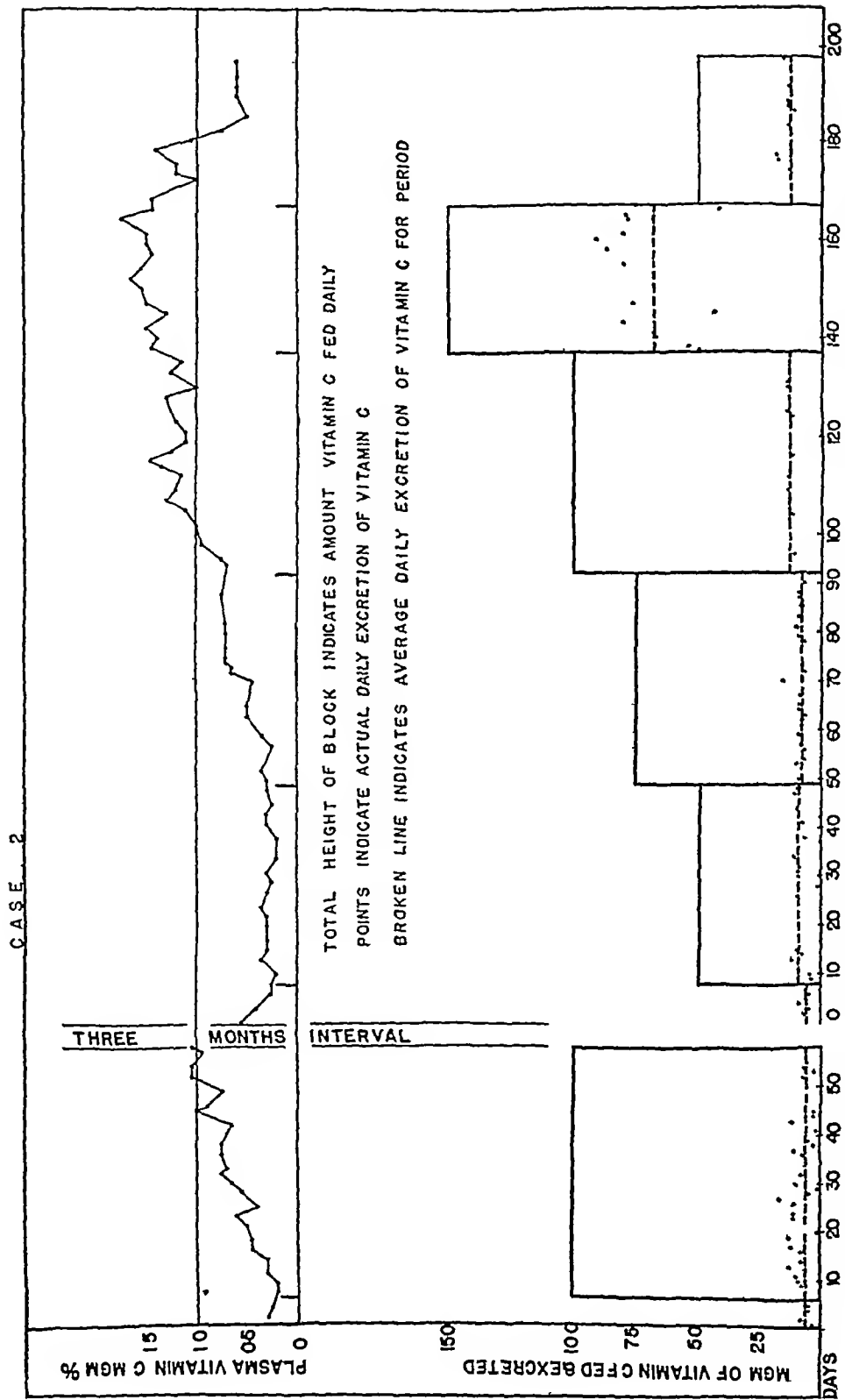


FIG 2 CASE 2 MG% OF VITAMIN C FED AND EXCRETED DAILY AND PLASMA LEVELS OF VITAMIN C DURING EACH PERIOD

the vitamin C status of the individual has been estimated on the basis of the urinary excretion of the vitamin. Obviously, the excretion of the vitamin depends on the amount ingested. However, until the ingestion exceeded 100 mgm of vitamin C daily, the excretion remained fairly constant and rarely exceeded 15 mgm of vitamin C in 24 hours. Therefore the 24-hour urinary excretion of vitamin C without any knowledge of the plasma level does not give complete information of the vitamin C status of the individual. An excretion of 15 mgm daily can occur at intakes varying from 50 to 100 mgm. of vitamin C and at fasting plasma levels ranging from 0.3 to 1.0 mgm per cent. This is shown in the summary of all the periods in all 3 cases (Figure 4), where the average daily excretion of vitamin C at intakes of 50, 75 and 100 mgm daily varied from 8 to 13 mgm. It is only when the intake exceeded 100 mgm. daily that there was any significant increase in the 24-hour urinary excretion of the vitamin. Below an intake of 100 mgm of vitamin C daily the plasma level was a more accurate index of the vitamin C intake. When the intake exceeded 100 mgm. daily the urinary excretion reflected more accurately the increases in the ingestion of the vitamin.

Apparently in the full-grown adult the daily vitamin C requirement is not dependent on age, height and weight. In these 3 cases the ages varied from 35 to 57 years, the heights from 65 inches to 72¼ inches and the weights from 135 to 160 pounds. Case 3 gained 20 pounds during the 200 days that he was under observation and this had no effect on the vitamin C requirement. For this reason we have not estimated the daily requirement on a per kilogram basis as was suggested by Heinemann (10).

SUMMARY

The daily excretion of vitamin C and the plasma concentration of the vitamin were determined on 3 male adults kept on control diets and fed varying amounts of vitamin C in the form of ascorbic acid.

When the amounts of vitamin C fed daily did not exceed 100 mgm the 24-hour excretion of vitamin C did not exceed an average of 13 mgm.

When more than 100 mgm was fed daily there was a sharp rise in the urinary excretion of the vitamin and the rise continued to parallel any increase in the ingestion of the vitamin.

When less than 100 mgm was fed daily, it was impossible either to raise or maintain a plasma vitamin C level of 1.0 mgm. per cent.

When fed 50 mgm. of vitamin C daily, the plasma concentration of vitamin C averaged 0.4 mgm. per cent. As the patients were normal and as there were no symptoms of vitamin C deficiency it is suggested that this plasma level be considered the lower limit of normal. At this plasma level, however, the body tissues are not saturated with vitamin C and saturation apparently does not occur until the plasma concentration is at a level of 1.0 mgm. per cent.

As a plasma concentration of 1.0 mgm. per cent can only be obtained and maintained on a daily intake of at least 100 mgm of vitamin C, it is suggested that this be considered the optimum daily intake.

BIBLIOGRAPHY

1. Smith, S. L., Human requirement of vitamin C. *J. A. M. A.* 1938, 111 1753.
2. Abt, A. F., Farmer, C. J., and Epstein, I. M., Normal cevamic (ascorbic) acid determinations in blood plasma and their relationship to capillary resistance. *J. Pediat.*, 1936 8, 1.
3. Rall, E. P., Friedman, G. J., and Rubin, S. H., Mechanism of excretion of vitamin C by human kidney. *J. Clin. Invest.*, 1938, 17, 765.
4. U. S. Department of Agriculture, Vitamin content of foods, Miscellaneous Publication, No. 275 June 1937.
5. Mindlin, R. L. and Butler, A. M., Determination of ascorbic acid in plasma: macromethod and micro-method. *J. Biol. Chem.* 1938 122 673.
6. Evelyn, K. A., Malloy, H. T. and Rosen, C., Determination of ascorbic acid in urine with photo-electric colorimeter. *J. Biol. Chem.*, 1938 126, 645.
7. (a) Van Werssch, H. J., Determinations of the daily requirements for ascorbic acid of man. *Acta brev. Neerland.*, 1936 6 86.
(b) Heinemann, M., Human Requirements for vitamin C. *Biochem. J.* 1936, 30 2299.
8. (a) Göthlin, G. F., Method of establishing vitamin C standard and requirement of physically healthy individuals by testing strength of their capillaries. *Skandinav. arch. f. Physiol.*, 1931 61 225.
(b) Göthlin, G. F., Human daily requirements of dietary ascorbic acid. *Nature*, 1934, 134 569.

reasonable, as a result of these observations, to reconsider our ideas of the normal plasma limit and to consider a plasma concentration of 0.4 mgm per cent as the low limit of normal. We do not wish to create the impression that this is a desirable plasma level of vitamin C for an individual. In fact these studies have shown that at this plasma level the tissues are not saturated and, when increasing amounts of vitamin C were fed, the plasma concentration continued to rise without any parallel increase in the excretion of the vitamin until the daily intake exceeded 100 mgm. Even when the plasma vitamin C level

was 0.8 mgm per cent the tissues were still apparently not saturated, as the excretion of vitamin C did not increase until a fasting plasma level of 1.2 mgm per cent had been reached. On the basis of these facts we would not recommend that a normal individual should only ingest enough vitamin C to maintain such a low plasma concentration. On the contrary we feel that *100 mgm of vitamin C daily* is the desirable intake and, if one expects to maintain a plasma concentration of 1.0 mgm per cent, this is the amount required.

In previous studies from many laboratories (9)

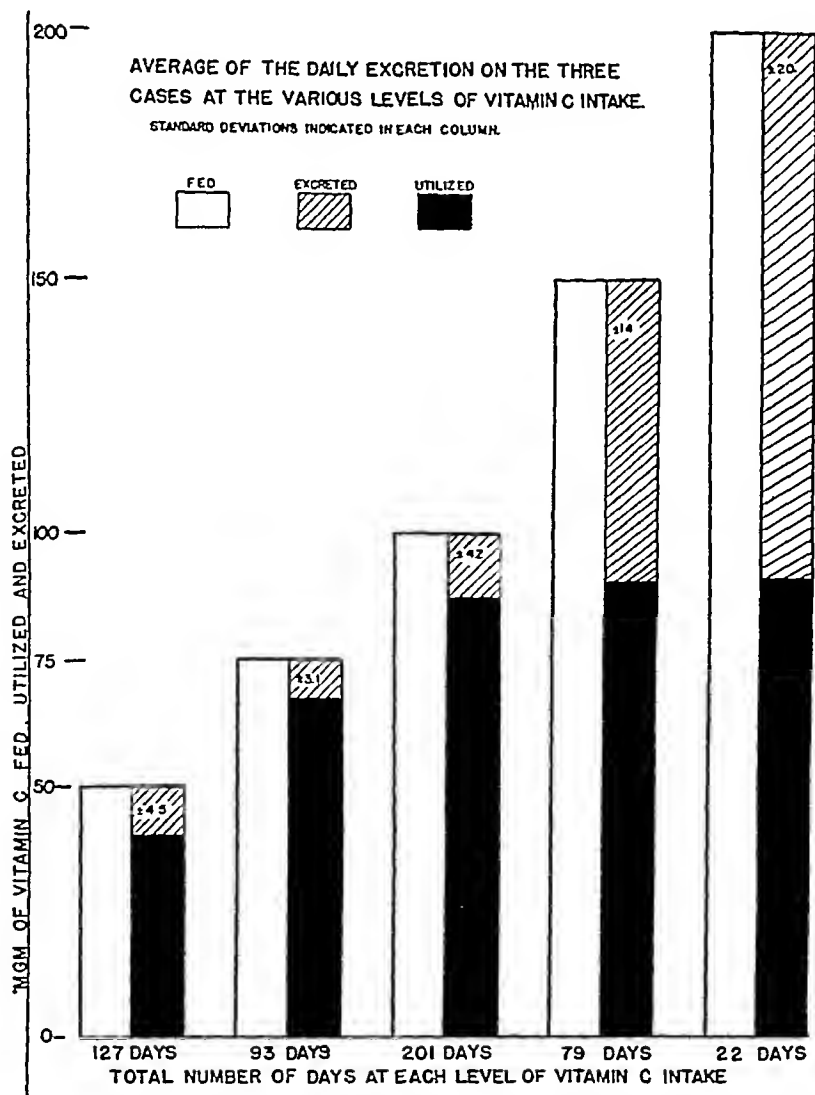


FIG. 4 AVERAGE OF THE DAILY EXCRETION ON THE THREE CASES AT THE VARIOUS LEVELS OF VITAMIN C INTAKE

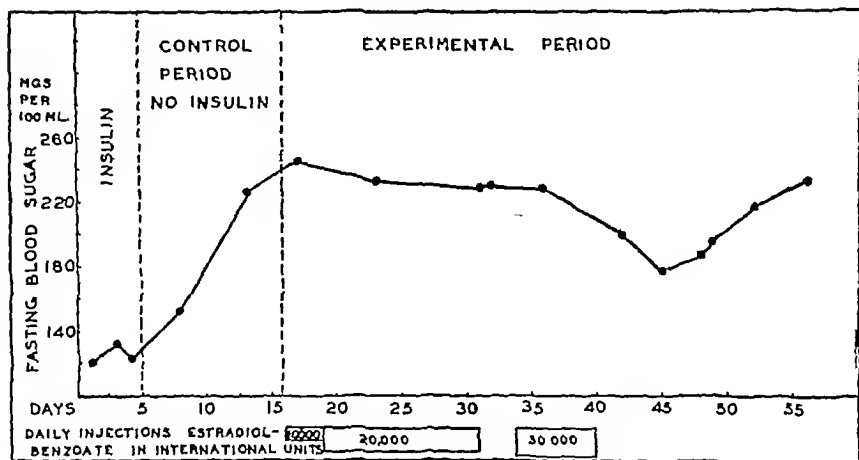


FIG. 1. RESULTS OF ESTRADIOLBENZOATE INJECTIONS IN PATIENT CASE 1

period of twenty two days. The fasting blood sugar values fell to about 140 mgm. per 100 cc. after fourteen injections. Further injections did not result in a significantly greater lowering. The fasting blood sugar remained around 140 mgm. per 100 cc. for nine days after the last injection of estradiolbenzoate, then started to rise and reached a level of 244 mgm. per 100 cc. on the sixty-sixth day.

The general condition of this patient was remarkably improved. She had suffered considerable pain from hypertrophic arthritis of both knees for one year. When first seen, she walked with difficulty. After the first four injections of estradiolbenzoate she volunteered the information that her arthritis had improved to such an extent that she could walk much better and could freely bend her right knee, which was previously stiff. Similar effects have been reported by Hall (15) in menopause arthralgia.

On the fifty-fifth day while the fasting blood sugar was going up the patient was discharged. Owing to her intelligence and cooperation it was possible to maintain the same diet at home and to continue the investigation. The results of the further observations are given in Figure 2B. On the sixty-sixth day 100,000 international units of estradiolbenzoate (2 cc.) were injected. This was followed the next day by metrorrhagia of three days duration. On the seventy-third day the patient received an injection of 50,000 international units of estradiolbenzoate (1 cc.) and from then on this amount was given twice a week. The fasting blood sugar again fell but more slowly than during the period of daily injections. From an initial level of around 240 it reached a value of 171 mgm. per 100 cc. on the ninety

fourth day, after seven injections. The patient then burned her hand and missed the next injection. One or both of these factors may have been responsible for the subsequent peak in the fasting blood sugar values (242 mgm. on the one hundred and first day). After the resumption of injections of 50,000 international units of estradiolbenzoate twice a week, the fasting blood sugar values again decreased and reached 155 mgm. per 100 cc. on the one hundred and twenty-second day after fifteen injections. On the one hundred and fifty-first day metrorrhagia set in and persisted in varying intensity for two weeks. The last injection of estradiolbenzoate was given on the one hundred and fifty-third day. The fasting blood sugar values then rose progressively and reached 263 mgm. per 100 cc. twenty-five days after the last injection.

Case 3 This 40-year-old white woman began to experience menstrual irregularities four years before entry to the hospital. Two years later a panhysterectomy was performed disclosing, according to a pathologist's report, late proliferating endometrium, early squamous cell carcinoma of the cervix, and thecal lutein cysts of the ovaries. On the day before operation she was found to have diabetes, which required 10 units of insulin and 15 units of protamine insulin daily for regulation when the patient entered the hospital for the present observations. As shown in Figure 3 during a control period of five days without insulin the fasting blood sugar averaged about 300 mgm. per 100 cc. Thereafter during the experimental period the patient was given fourteen daily injections of 50,000 international units of estradiolbenzoate each. After five days the fasting blood sugar aged about 240 mgm. per 100 cc. for nine

absolutely no effect on human diabetes. They used daily doses varying between 100 and 400 rat units. Glen and Eaton (12) improved the condition of a severely diabetic woman, in whom the diabetes apparently started after an ovariectomy, by injections of estrogenic substance. In this case there was said to be an increased amount of diabetogenic hormone in the blood, as tested by the method of De Wesselow and Griffiths (13).

One possible explanation for the conflicting reports in the literature may be the present inability to discriminate between those diabetics in whom the pituitary may have played an etiological rôle and those with purely pancreatic diabetes. If estrogenic substance can exert an influence on diabetes, such an effect theoretically should be demonstrable more easily at a life period when the organism is lacking in ovarian estrogenic hormone. With this in mind, it was decided to study the effect of injected estrogenic substance on the blood sugar of diabetic women who had passed the menopause.

METHODS

Five female diabetic patients who had passed the menopause were chosen for study. Two of these women (Cases 1 and 2) gave an unequivocal history of the simultaneous onset of the menopause and diabetes. In Case 3 menopause symptoms preceded by two years the detection of diabetes at the time of a panhysterectomy. In Case 4 the correlation was difficult to ascertain, but it is probable that the diabetes began shortly before the menses became irregular, amenorrhea occurring four years later. In Case 5 the menopause preceded the sudden onset of diabetes by seven years.

The general experimental plan was as follows. In each instance the patient was maintained throughout the entire period of observation on a diet containing 151 grams of carbohydrate, 74 grams of protein and 92 grams of fat, with a caloric value of 1,728 calories. This diet was tolerated well and in no instance was there any significant change in weight during the period of observation. During a first period the patients were treated with insulin in addition to the basal diet. During the next control period, varying from five to fourteen days, no insulin was given

and the fasting blood sugar values rose. During an immediately subsequent experimental period, intramuscular injections of estradiolbenzoate (Progynon B)³ in amounts up to 50,000 international units (10,000 rat units) were administered daily or otherwise, as shown in Figures 1 to 5. Throughout the entire period of observation the fasting blood sugar values were determined at frequent intervals on venous blood (Folin method). For convenience the experimental results in each case are discussed separately.

In 2 patients an attempt was made to determine whether they belonged, according to the method of Himsworth (14), to insulin-sensitive or insulin-insensitive types, respectively. In 4 patients urine assays for follicle-stimulating pituitary hormone were made both before and after the period of injection of estradiolbenzoate. Finally, the influence of these injections on the basal metabolic rate was determined in two instances.

RESULTS

Case 1 This 50-year-old white woman was found to have diabetes at the time of the menopause, six months previous to admission to the hospital. The diabetic condition was controlled by 30 units of protamine insulin a day. The results of this observation are given in Figure 1. During the eleven-day control period without insulin the fasting blood sugar values rose. During the first three days of the subsequent experimental period daily injections of 10,000 international units (1 cc.) of estradiolbenzoate were given, without any effect on the fasting blood sugar. The daily dose was then increased to 20,000 international units (2 cc.) and continued at this level for twelve days, again with no effect on the fasting blood sugar values. After an interval of four days, 50,000 international units of estradiolbenzoate (1 cc.) were injected daily for six days. The fasting blood sugar values then decreased from around 230 mgm. and reached the lowest value of 178 mgm. per 100 cc. six days after the last injection of estradiolbenzoate. The fasting blood sugar values then slowly rose.

Case 2 This 62-year-old white woman developed diabetes within three months after the menopause, fourteen years before admission to the hospital. The diabetic condition was controlled by 35 units of protamine insulin a day. The results of this observation are given in Figures 2A and 2B. During the control period of thirteen days without insulin the fasting blood sugar values rose to 267 mgm. per 100 cc. During the following experimental period daily injections of 50,000 international units (1 cc.) of estradiolbenzoate were given over a

³ We wish to extend our thanks to the Schering Corporation for a generous supply of Progynon B.

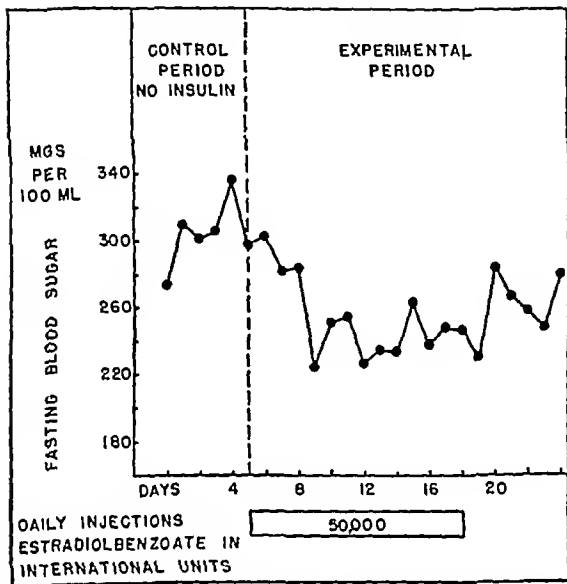


FIG. 3. RESULTS OF ESTRADIOLBENZOATE INJECTIONS IN PATIENT CASE 3

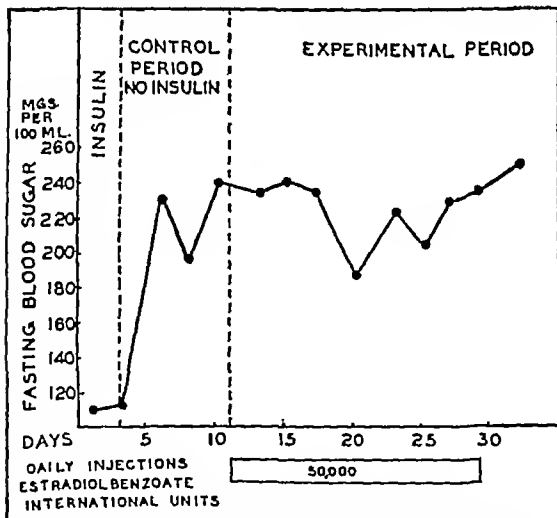


FIG. 4. RESULTS OF ESTRADIOLBENZOATE INJECTIONS IN PATIENT CASE 4

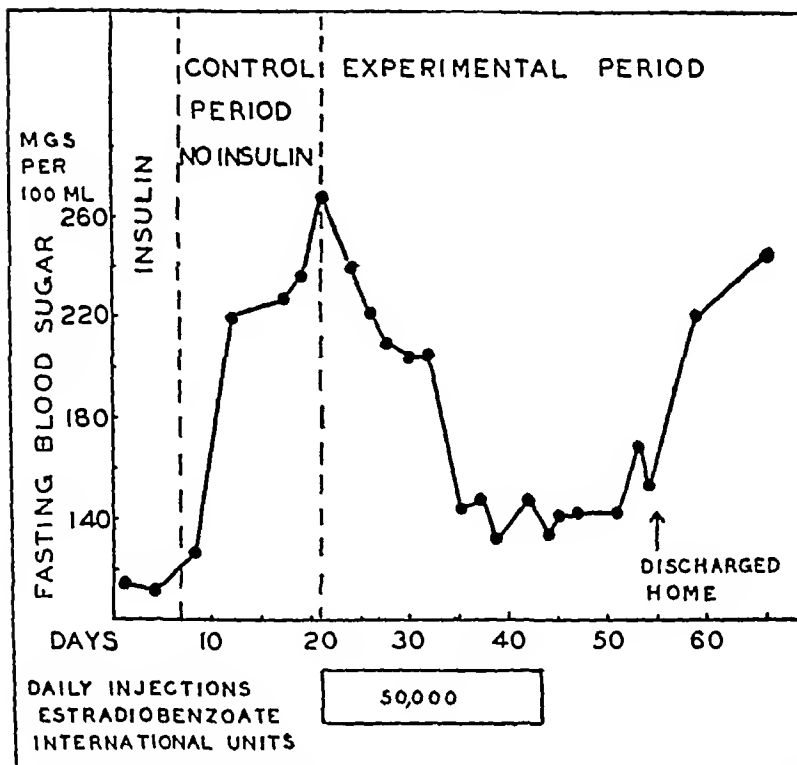


FIG 2A RESULTS OF ESTRADIOLBENZOATE INJECTIONS IN PATIENT CASE 2

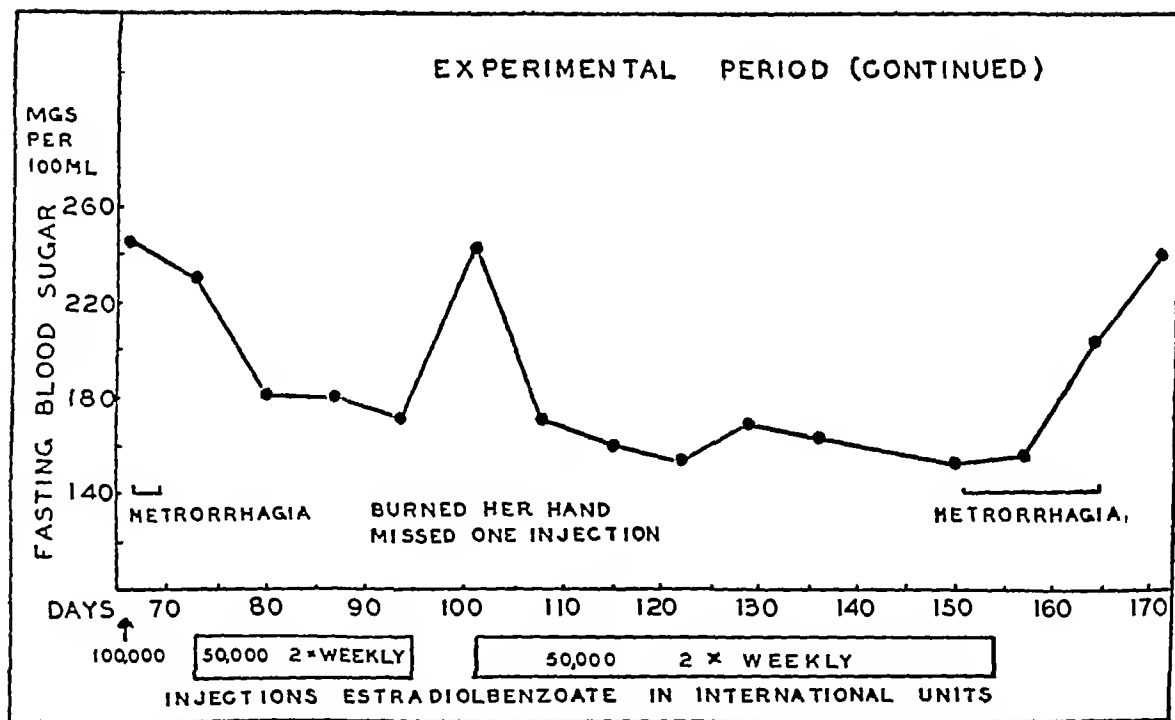


FIG 2B RESULTS OF ESTRADIOLBENZOATE INJECTIONS IN PATIENT CASE 2, CONTINUED

tive group, while case 4 was of the insulin sensitive type

Urine assays for follicle stimulating hormone
Tests for follicle stimulating hormone were performed on twenty four hour urine specimens from Cases 1 2 4 and 5 both before and after injections of estradiolbenzoate. The method used was Zondek's alcohol precipitation test using rats as the test animal⁴. With the particular technique employed negative tests (less than 20 rat units per liter) are uniformly obtained in normal women before the menopause, and positive tests (at least 40 rat units per liter) are obtained in most cases after the menopause. In each of our 4 patients a positive test was obtained before the injections of estradiolbenzoate and a negative test was obtained two days after the last injection. These results indicate that the injections of estradiolbenzoate were effective in inhibiting the follicle stimulating function of the anterior pituitary.

Influence of the injection of estradiolbenzoate on the basal metabolism In Cases 2 and 4 the basal metabolic rate was measured before and after injection of estradiolbenzoate. In each instance the initial basal metabolic rate was normal or slightly low. In no instance did a significant change occur after the injections.

DISCUSSION

Urine assays in 4 of 5 diabetic women who had passed the menopause and who were given injections of 50 000 international units of estradiolbenzoate for several days indicated the effectiveness of this material in inhibiting the follicle-stimulating function of the anterior pituitary. In 2 patients Cases 1 and 2, in whom the diabetes began at the time of the menopause, the elevated fasting blood sugar values which were reached after the discontinuance of insulin therapy were significantly lowered by daily injections of 50 000 international units of estradiolbenzoate. Moreover, a few days after the last injection the fasting blood sugar values went up again. In the patient, Case 3 in whom diabetes appeared two years after the menopause began and at the time of surgical removal of the ovaries it is probable

that a similar effect was obtained. The insistence of the patient on leaving the hospital, however, unfortunately prevented a sufficiently long period after the termination of the injections in which to observe a potential rise of the blood sugar. In the patient, Case 4 in whom the diabetes was discovered shortly before the menopause, no significant effect on the blood sugar was observed. In the patient, Case 5, in whom the menopause preceded the onset of the diabetes by at least seven years there was no lowering of the blood sugar as a result of injections of estradiolbenzoate.

The blood sugar lowering effect is considered to have been due to inhibition of the diabetogenic factor of the anterior pituitary by the injected estradiolbenzoate. It is not necessarily an argument against this assumption that although before the injection of estradiolbenzoate 4 of these 5 patients showed increased excretion of follicle stimulating hormone the blood sugar of only 3 responded significantly to estradiolbenzoate. Although estradiolbenzoate has been shown to be capable of lowering the elevated basal metabolic rate of women who have become thyrotoxic after the menopause (4), in the present observations the normal basal metabolic rate remained unaffected. Apparently the inhibitory effect of estradiolbenzoate is manifest only in the presence of excessive secretion of thyrotropic hormone of pituitary origin. Similarly, in the present observations the inhibitory effect of estradiolbenzoate in 3 of the patients may perhaps be taken to indicate an excessive influence of pituitary diabetogenic factor.

In future it may be possible by means of tests for diabetogenic factor in the blood or urine (13) (16), to distinguish between two types of diabetes, one mainly pancreatic the other mainly pituitary in origin. According to Himsaworth's (14) technique, Case 2 belonged to the insulin insensitive group while Case 4 was apparently of the insulin-sensitive type. The conflicting results in the literature on the subject of the effectiveness of estradiolbenzoate in diabetes may be due either to such differences in the type of diabetics studied or to the fact that insufficient amounts of estrogenic substance have been used. The negative results obtained by Collens and his associates (11) can certainly be explained on this basis, because they injected only 2 000 international units daily, whereas the observations on C

⁴The tests were performed by Miss Estelle Donovan and Miss Priscilla Spalding of the Aschheim Zondek Laboratory of the Mallory Institute for Pathology of the Boston City Hospital.

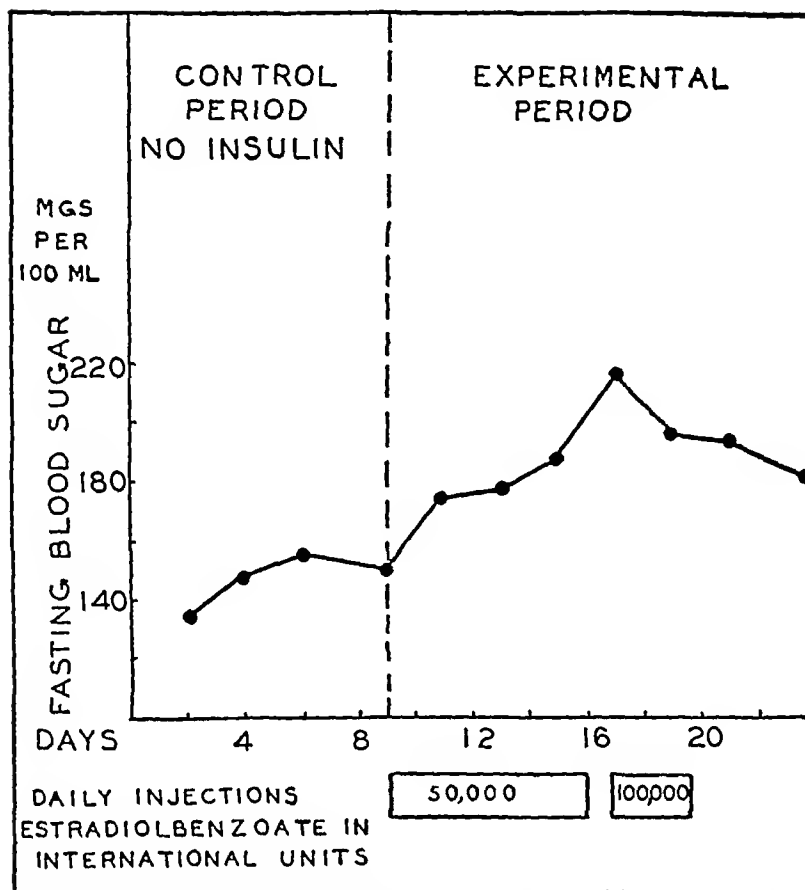


FIG 5 RESULTS OF ESTRADIOLBENZOATE INJECTIONS IN PATIENT CASE 5

when the injections were discontinued, the data suggest that the fasting blood sugar was a little higher than during the injection period.

Case 4 This 54-year-old white woman apparently became diabetic eight years before entry to the hospital and shortly before the menses became irregular. Amenorrhea occurred four years later. The diabetic condition was controlled by 30 units of protamine insulin a day. The results of this observation are given in Figure 4. During the seven-day control period without insulin the fasting blood sugar values rose and finally reached 240 mgm. per 100 cc. During the following experimental period daily injections of 50,000 international units of estradiolbenzoate (1 cc.) were given over a period of eighteen days. Despite considerable fluctuations during both the control and the experimental periods, the injected estradiolbenzoate had no consistent effect on the fasting blood sugar, which was 239 mgm. per 100 cc. on the thirty-second day after eighteen injections.

Case 5 This 58-year-old white woman experienced the menopause more than ten years before entry to the hospital. Seven years later she rather suddenly developed symptoms which led to the diagnosis of diabetes. This was controlled during the last two years with 25 units of insulin daily. The results of the observations are given

in Figure 5. During a nine-day control period without insulin her fasting blood sugar averaged about 150 mgm per 100 cc. During the subsequent experimental period daily injections of 50,000 international units for eight days and of 100,000 international units of estradiolbenzoate for three additional days did not prevent further increases in the fasting blood sugar level, which reached a maximum of 217.7 mgm per 100 cc. on the ninth day of the experimental period.

Glucose tolerance Glucose tolerance tests in our patients gave typical diabetic curves. Fifty grams of glucose were given orally instead of breakfast, samples of venous blood were taken every half hour for two or three hours, the patient remaining in bed during the test. An attempt was made in two of our cases to differentiate them according to Himsworth (14) into insulin-sensitive and insulin-insensitive types, using the glucose tolerance test with and without simultaneous intravenous injection of insulin. Case 2 apparently belonged to the insulin-insensi-

POSTMORTEM HEPATIC GLYCOGENOLYSIS IN HYPERINSULINISM AND GLYCOGEN DISEASE¹

By H P G SECKEL

(From the Department of Pediatrics University of Chicago Chicago)

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The fundamental pathology of von Gierke's glycogen disease (1) lies in the inhibition of *in vivo* and postmortem glycogenolysis of the liver and other organs. In the discussion of the pathogenesis of this disorder, hyperinsulinism has been time and again suggested since Wilder and his collaborators (2) described their famous case of pancreatogenic hyperinsulinism with severe spontaneous hypoglycemia and a very high liver-glycogen content. They hunted at a similar situation in the equally famous case of Parnas and Wagner (3). This case, concerning a 9-year-old girl with hepatomegaly hypoglycemia ketonuria and retardation of growth, is now almost universally recognized as the first case of glycogen disease ever described in the literature before the final elucidation of the disorder by von Gierke in 1929 (1) (cf. the early case of Worster Drought (4a) and especially that of Snapper and van Creveld (5)). It has been noticed however, that in Wilder's case of carcinoma of the Langerhans islets with metastases to the liver, postmortem hepatic glycogenolysis was not determined and as a matter of fact has never yet been determined in the numerous cases of hyperinsulinism reported in the literature. It is the purpose of this communication to fill this gap by examining postmortem hepatic glycogenolysis in 2 cases of spontaneous hypoglycemia, one of which is almost certainly due to pancreatogenic hyperinsulinism while the other will be tentatively classified as 'neurogenic hyperinsulinism'.

CASE REPORTS²

Case 1. Albert R. 36 years, Number 195034 Admitted to the Department of Neuro-Surgery University of Chicago on March 21 1938

The patient had unexplained attacks of complete unconsciousness for quite some time which were revealed

¹ This work was aided by a grant from the Douglas Smith Foundation for Medical Research.

² For more elaborate findings see later publication by N B Friedman and W Sweet to whom the writer is greatly indebted for communicating the data.

to be typical seizures of spontaneous hypoglycemia. Blood sugar on fasting and during attacks was between 16 and 23 mgm. per cent no glycosuria no ketonuria. The liver was found to be grossly enlarged and nodular to palpation. The hypoglycemic seizures were immediately stopped or prevented by intravenous glucose infusions but not by adrenalin injections. The daily glucose requirement by mouth and parenterally was about 600 grams. Under this regime, further hypoglycemic seizures were prevented the body weight increased from 57.1 to 64.3 kgm. within three months in spite of the underlying carcinoma. Alimentary glucose tolerance test high rise of blood sugar falling off very early to hypoglycemic levels. Adrenalin test (1 mgm. subcutaneously) non fasting blood sugar 73 mgm. per cent, after 20 minutes 98 mgm. per cent, after 45 minutes 65 mgm. per cent slight vasomotor response. There was no abnormal sensitivity to insulin. The blood cholesterol was normal.

Clinical diagnosis. Spontaneous hypoglycemia, probably due to a pancreatic tumor. An exploratory laparotomy was done on April 15th. Innumerable white metastatic nodules were seen all over the liver. Following the ether anesthesia, the glucose requirement was considerably reduced for several days. During 24 hours preceding death, 580 grams of glucose were administered parenterally the last dosage about 2 hours before death. Exitus on June 22, 1:20 p.m.

Autopsy (started 15 minutes after death). Carcinoma of the pancreatic islets with nodular metastases to the liver and other viscera. Histologically there was no abnormal glycogen storage outside the liver and muscles. An insulin assay of the liver nodules was unsatisfactory.

Case 2. William M., 56 years, Number 206024 Admitted to the Department of Medicine University of Chicago, on September 23 1938.

The patient had supposedly uremic attacks with complete unconsciousness for about 8 weeks which were recognized as typical seizures of spontaneous hypoglycemia. Blood sugar on fasting and during attacks was between 26 and 34 mgm. per cent, occasionally higher (72 mgm. per cent) no glycosuria no ketonuria. The hypoglycemic seizures were stopped and prevented by administration of large amounts of glucose by mouth and parenterally. Alimentary glucose tolerance test High rise of blood sugar for about an hour falling off to around 30 mgm. per cent after 2½ hours. Adrenalin tests (1 mgm. subcutaneously) fasting blood sugar 62 mgm. per cent, after 5 15 and 60 minutes 58 60 and 65 mgm. per cent respectively 3 mgm. intravenously non-fasting blood sugar 76 mgm. per cent, after 5 10 and 40 minutes 84

daily injections of even 10,000 and 20,000 international units, respectively, were ineffective in lowering the blood sugar

The type of patient most likely to respond to estradiolbenzoate would theoretically be the case in which the diabetes appeared with or shortly after the menopause, or shortly after the surgical removal of the ovaries. The activity of estradiolbenzoate in such patients would be expected to be most readily observed during or soon after the menopause. It is not the purpose of this article to discuss the nature of the diabetogenic factor in the anterior pituitary. Data on this subject can be found in the papers by Young (17) (18), Long (19), Houssay (1), Elmer, Giedosz and Scheps (20), Neufeld and Collip (21) and Taubenhaus (22).

SUMMARY

Five diabetic women who had passed the menopause were given intramuscular injections of estradiolbenzoate (50,000 international units daily). In the four instances tested, the excretion of follicle-stimulating hormone of the pituitary was significantly decreased by this procedure. A significant lowering of the fasting blood sugar resulted in 2 of the patients (Cases 1 and 2) in whom the onset of diabetes coincided with the menopause, and probably in a third (Case 3) in whom the menopause preceded the diabetes. It is suggested that this effect was due to inhibition by the injected estradiolbenzoate of a postmenopausal excessive production of the pituitary diabetogenic factor. In the other 2 patients (Cases 4 and 5), there was clearly no effect from the estradiolbenzoate injections. In Case 4 the onset of diabetes preceded the menopause and in Case 5 the menopause preceded the onset of diabetes by at least seven years.

BIBLIOGRAPHY

- Houssay, B A., Hypophysis and metabolism. *New England J Med.*, 1936, 214, 961
- Young, F G., Permanent experimental diabetes produced by pituitary (anterior lobe) injections. *Lancet*, 1937, 2, 372.
- Albright, F., Studies on ovarian dysfunction, menopause. *Endocrinology*, 1936, 20, 24
- Hoet, J P, and Gessler, C., Pathologie van de schildklier. *Vlaamsch Geneesk. Tijdschrift*, 1937, 17, 281
- Barnes, B O, Regan, J F, and Nelson, W O, Improvement in experimental diabetes following the administration of amniotin. *J A. M A.*, 1933, 101, 926
- Nelson, W O, and Overholser, M D, Effect of oestrogenic hormone on experimental pancreatic diabetes in monkey. *Endocrinology*, 1936, 20, 473
- Rathery, F, and Rudolf, M, Folliculine, insuline et diabete. *Bull et mém. Soc. méd. d. hop de Paris*, 1928, 52, 741
- Carnot, Terris, and Caroli, Un cas de "diabète ovarien," résistant a l'insuline, tres amélioré par la folliculine. *Bull. et mém Soc. méd. d. hop de Paris*, 1928, 52, 738
- Mazer, C, Meranze, D R, and Israel, S L, Evaluation of constitutional effects of large doses of estrogenic principle. *J A. M A.*, 1935, 105, 257
- Jones, M S, and MacGregor, T N, Inhibitory effect of follicular hormone on anterior pituitary in humans. *Lancet*, 1936, 2, 974
- Collens, W S, Slo-Bodkin, S G, Rosenbliett, S, and Boas, L C, Effect of estrogenic substance on human diabetes. *J A M A*, 1936, 106, 678
- Glen, A., and Eaton, J C, Insulin antagonism. *Quart. J Med.*, 1938, 7, 271
- De Wesselow, O L V, and Griffiths, W J, On possible role of anterior pituitary in human diabetes. *Lancet*, 1936, 1, 991
- Himsworth, H P, Diabetes mellitus, its differentiation into insulin-sensitive and insulin-insensitive types. *Lancet*, 1936, 1, 127
- Hall, F C., Menopause arthralgia, study of 71 women at artificial menopause. *New England J Med.*, 1938, 219, 1015
- Byerling, T., Investigation of diabetogenous hormone in urine. *Acta med Scandinav.*, 1938, 94, 483
- Young, F G, Glycogen and metabolism of carbohydrate. *Lancet*, 1936, 2, 237 and 297
- Young, F G, Experimental investigations on relationship of anterior hypophysis to diabetes mellitus. *Proc. Roy Soc. Med.*, 1938, 31, 1305
- Long, C N H, Disturbances of endocrine balance and their relation to diseases of metabolism. *Ann. Int. Med.*, 1936, 9, 1619
- Elmer, A. W, Giedosz, B, and Scheps, M, Anterior pituitary and its diabetogenic and pancreatropic (blood sugar decreasing) activity. *Acta med. Scandinav.*, 1937, 93, 487
- Neufeld, A. H, and Collip, J B, Studies of effects of pituitary extracts on carbohydrate and fat metabolism. *Endocrinology*, 1938, 23, 735
- Taubenhaus, M, Untersuchungen über das Kohlehydrat- und Fettstoffwechsel-Hormon der Hypophyse bei Diabetikern und bei Hypophysentumoren. *Wien Arch. f inn Med*, 1936, 29, 251

of the rectus muscle (0.52 gram per cent), although smaller than in Case 1, was still higher than might have been expected 2½ hours after death. Rather large amounts of glycogen were found in the liver not only at autopsy (3.52 grams per cent), but especially at biopsy (6.1 grams per cent). The latter finding is all the more remarkable as the biopsy specimen was taken after a long ether anesthesia which is known to mobilize glycogen from the liver (14), even in cases of pancreatic hyperinsulinism (2). In contrast to Case 2 in Wilder's patient the liver glycogen was lower during ether anesthesia (3.49 grams per cent) than 2½ hours after death (8.25 grams per cent). The

amount and time of glucose administrations during the day preceding operation or death may well play a part in determining the glycogen figures of such cases.

The curves of postmortem hepatic glycogenolysis of Cases 1 and 2 as presented in Table I and Figure 1 varied greatly according to the temperature applied. At 37° C, glycogen dissimilation proceeded constantly and rapidly and was almost completed within 1 or 2 days. At ice box temperature, it proceeded much slower and more irregularly after 6 days, about 50 per cent of the glycogen was still present. Since, as yet, the writer was unsuccessful in securing control figures

TABLE I
Glycogen content and postmortem glycogenolysis of various unsliced tissues
(glycogen in grams per 100 grams of fresh tissue)

Tissues	Case 1 Experiment 171 24 minutes post mortem		Case 2 Experiment 173 2 hours 40 minutes post mortem		Normal Rats			Rab- bit's Num- ber 189	Glycogen Disease				Neutro- boon (34)††			
					Experiment 36		Experiment 173a		Schön- heimer (26)††		Uns- helm (27)††			van Creveld (8)††	Harris (28)††	
	37 C.	Ice box	37 C.	Ice box	37 C.	37 C.			Ice box	37 C.	37° C.	Ice box			37° C.	Ice box
Biopsy																
Blood	0.014															
Liver*			6.10									0.018				
Autopsy																
Tumort			0.05													
Mesent. fat	0															
Muscle	1.57		0.52											5.47	0.32	
Heart†	0													7.90	0.40	
Liver	1.58	1.58	3.52	3.52	6.91	5.69	5.69	11.44	10.43	14.20	9.13	4.12	5.99			
30 Min					4.08	4.08	5.19									
60 Min			3.00	3.22				9.55								
90 Min					4.09	2.36	4.90									
2 Hrs				3.11	3.46			8.07								
3 Hrs	1.11	1.17	2.40	3.07	2.95	0.94	4.60	8.22								
6 Hrs.	0.76	0.83	2.12	2.91	3.06	0.38	4.05	7.50								
18 Hrs.	0.22	0.88														
24 Hrs	0.07	1.03	0.95	2.83	0	0.07	3.27	2.24							4.19	
2 Days		0.94	0.42	2.46			2.50					6.74 Heart				
3 Days		0.81		2.13												
4 Days				2.00												
5 Days		0.67														
6 Days		0.65		1.95					10.43	13.70		3.00				
7 Days							0.83									

* Determination by Dr. R. Sternheimer, Department of Medicine.

† Tumor kept on ice for 6½ hours before examination.

† Heart of Case 1 examined about one hour after death.

† This animal received 5 grams glucose daily in addition to the stock diet.

† von Gierke's case of Hepatomegalia Glycogenica autopsy 24 hours postmortem. Liver kept on ice for 4 additional days before the first glycogen determination was made by Schoenheimer.

† Case of Hepatomegalia Glycogenica autopsy and glycogen determination 24 hours after death. Liver kept in ice box as pulp.

** Case of Cardiomegalia Glycogenica autopsy and glycogen determination 24 hours postmortem.

†† Case of Cardiomegalia Glycogenica heart kept on ice for 17 hours, muscle for 2 days and liver for 12 days after death. At ice box temperature heart glycogen decreased very little within 6 days.

††† Rabbit operated upon thoracic nerve segments (see text) start of glycogen determinations 5 hours after death.

88, and 78 mgm per cent, respectively, intensive vasomotor response. Three mgm. adrenalin intravenously did not arouse the patient from hypoglycemic coma. Blood cholesterol was 185 mgm per cent. The liver was not enlarged to palpation. On x-ray examination a marked elevation of the right leaf of the diaphragm with corresponding compression atelectasis of the right lung was found.

Clinical diagnosis Spontaneous hypoglycemia, presumably from a pancreatic adenoma. On October 17th, an exploratory laparotomy was done under spinal and ether anesthesia. No pancreatic tumor was discovered, the liver appeared to be normal, a biopsy was taken for chemical analysis. Postoperatively, 4 times 75 grams of glucose were administered parenterally without arousing the patient from unconsciousness. Exitus next morning at 8:15.

Autopsy (started at 10 a.m.) Massive fibroma on right top of the liver bulging the medial aspect of the right diaphragm, pancreas normal, no metastases anywhere. No autopsy of the brain was made. Histologically, no glycogen was stored except in liver and muscles. An insulin assay of the fibroma did not reveal any insulin.

Chemical analyses

Glycogen was determined in various organs with Plueger's method as modified by Good, Kramer and Somogyi (6). The final estimation of glucose was made with Somogyi's modification (7) of Shaffer and Hartmann's method (factor 0.927). Blood glycogen was estimated with van Creveld's method (8a). All figures are based on duplicate determinations. Small pieces of tissue were put into dry ice immediately after the organs had been received at autopsy, i.e., 24 minutes after death in case 1, and 2 hours and 40 minutes after death in case 2. In the animal experiments performed for control purposes, tissue pieces were put into dry ice 2 to 3 minutes after death. In both the human and animal experiments, *unliced* pieces of one and the same liver lobe were preserved in a wet chamber, either at 37° C with toluene added to prevent bacterial growth, or at ice box temperature with no preservative added. The glycogen content was determined at various intervals in the liver tissue in order to obtain time curves of post-mortem hepatic glycogenolysis. In Table I, the figures observed in the 2 clinical cases of hyperinsulinism are listed in conjunction with corresponding determinations in a few cases of glycogen disease reported in the literature as well as in normal rats and a rabbit. Liver glycogenolysis curves of the 3 categories are presented in Figures 1 and 2.

In both clinical cases of hyperinsulinism *liced* liver specimens were suspended for 2 hours at 37° C in a phosphate-buffered salt solution at pH 7.4 (for technique see Seckel (9a)). The following glycogen figures were observed in the liver slices. Case 1. At start (60 minutes after the liver was received) 1.37 grams per cent, after 60 minutes 0.65 gram per cent, after 120 minutes 0.53 gram per cent. By adding 1 per cent conjugated

bile salts to the suspension solution, as has been done earlier with rat liver slices (Seckel, 9a), the 60 minutes glycogenolysis was increased to 0.396 gram per cent, 0.1 per cent bile salt and 4 units of insulin per cc. of solution proved to be ineffective. Case 2. At start (60 minutes after the liver was received) 3.0 grams per cent, after 60 minutes 2.81 grams per cent, after 120 minutes 1.85 grams per cent.

Finally, the proportion of lyo- and desmo-glycogen was determined in the two human livers by repeated extractions with boiling water according to Willstätter and Rohdewald's method (10). Liver pieces kept in the ice box for 6 days were used for this purpose. In Case 1, 20.7 per cent of the total liver glycogen was found to be desmo-glycogen and 79.3 per cent to be lyo-glycogen. In Case 2, the corresponding figures were 14.4 and 85.6 per cent. In a normal rat liver, 18 and 82 per cent were found respectively (Experiment Number 129). In fresh geese livers, Willstätter and Rohdewald found 10 to 13 and 87 to 90 per cent, respectively.

COMMENT

In Case 1, the blood glycogen figure was within normal limits (average for normal children 12.9 mgm per cent (8a)). No glycogen was found in the mesenteric fat. In Schonen's (11) experiments in young dogs fattened on "glycogen mast," the fat contained up to 8.8 grams per cent glycogen. Probably the patient's heart was glycogen-free because of the delay in chemical examination. The glycogen content of the rectus abdominis muscle (1.57 grams per cent) seems to be unusually high. In view of the large amount of glucose administered to the patient, the liver-glycogen content of 1.58 grams per cent appears to be rather low. However, it is still considerably above the indirect estimations of human liver glycogen at the moment of a "chronic death" as carried out by Popper and Wozasek (12a) and Burghard and Paffrath (13) (average of 0.65 gram per cent for "total carbohydrates", for "glycogen" subtract 0.23 gram per cent). Furthermore, this patient's liver was filled with histologically glycogen-free metastatic nodules and was also invaded with tiny cell nests throughout those seemingly "normal" portions which were used for chemical examination. This not only accounts for the comparatively low glycogen content of the organ but also for the gross aberrations of individual figures in the curve of hepatic glycogenolysis (Table I, ice box temperature, 6- and 18-hour samples).

In Case 2, the liver fibroma was almost glycogen free on chemical analysis. The glycogen content

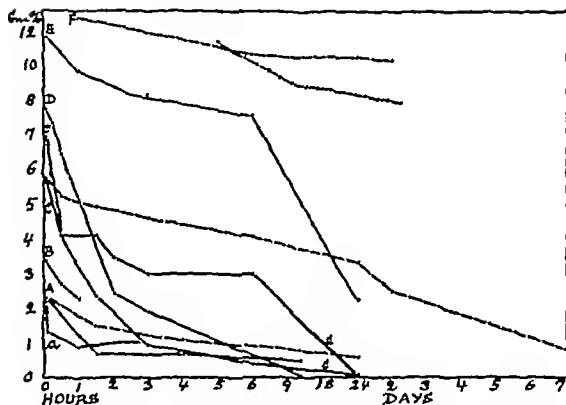


FIG. 2. TIME CURVES OF POSTMORTEM HEPATIC GLYCOGENOLYSIS OF NORMAL EXPERIMENTAL ANIMALS

———— Intact tissue at 37 C. - - - - - Intact tissue at ice box temperature. ——— Pulped tissue at ice box temperature. *a* = Cat (Evans *et al* at "room temperature") *A* = Goat (Burghard and Paffrath) *B* = Macacus Rhesus (Burghard and Paffrath 17 C.) *C* = Rat (Number 173 a, Table 1) *C'* = Rat (Number 36, Table 1) *D* = Guinea Pig (Popper and Worasek Number 3 sliced tissue, phosphate buffer pH 6.9) *E* = Rabbit (Number 189 Table 1) *F* = Rabbit (Popper and Worasek Number 2 in the lower curve, the liver was kept at 37 C. for 4 hours before being transferred to ice box temperature phosphate buffer pH 6.9)

(Figure 2 *F*) Mild hypoglycemia in spite of high liver-glycogen content was found in young dogs fed on "glycogen mast" (16)

DISCUSSION

There can be hardly any doubt that in the first case of spontaneous hypoglycemia we were dealing with a pancreatogenic hyperinsulinism originating from a carcinoma of the Langerhans islets with insulin producing metastases to the liver and other organs. In Case 2, this pathology was clearly excluded thus leaving room for speculation as to the nature of a spontaneous hypoglycemia associated with a massive fibroma on top of the liver. Most probably the anatomical localization of the liver tumor gives the clue to a satisfactory explanation. The right side fibroma may have exerted an immediate pressure on the right splanchnic nerves and the right celiac ganglion, thus blocking the sympathetic impulses to the liver. Such impulses are known to mobilize glycogen

from the liver either by direct nervous stimulation (*eg* piqure) or by indirect stimuli such as hypoglycemia (17) or adrenalin supply. If the splanchnic nerves are cut in cats, a decrease in blood sugar and an inhibition of glycogenolysis of surviving liver slices ensue (Evans Tsai and Young (14)). This indicates a relative preponderance of antagonistic impulses such as insulin supply, *ie* relative neurogenic hyperinsulinism.

The following conclusions may be drawn from the chemical investigations presented in the 2 cases of spontaneous hypoglycemia, the one most probably due to pancreatogenic hyperinsulinism the other possibly due to neurogenic hyperinsulinism.

(1) More than normal amounts of glycogen were accumulated in the liver and muscles, hypoglycemia, therefore, was not due to lack of glycogen depots.

(2) Liver glycogen was not abnormally fixed as so-called desmo-glycogen therefore, hypoglycemia was not caused in this manner.

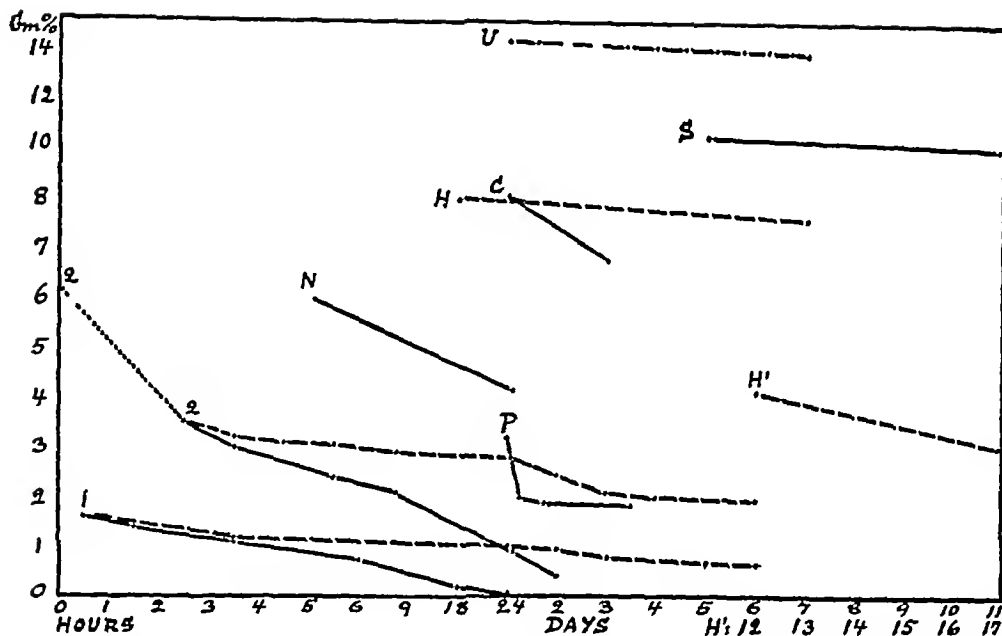


FIG 1 TIME CURVES OF POSTMORTEM GLYCOGENOLYSIS OF VARIOUS DISEASED TISSUES
(cf TABLE I)

———— Intact tissue at 37° C - - - - Intact tissue at ice box temperature.
 Pulped tissue at ice box temperature 1 = Liver of Case 1, pancreatogenic hyperinsulinism 2 = Liver of Case 2, "neurogenic" hyperinsulinism (dotted line between biopsy and autopsy) P = Liver of Popper and Wozasek's insulin-treated infant with diarrhea (sliced tissue, phosphate buffer, pH 6.9) S = Liver of Schoenheimer-von Gierke's case of glycogen disease. U = Liver of Unshelm's case of glycogen disease. C = Heart of van Creveld's case of glycogen disease. H, H' = Heart and liver, respectively, of Hertz' case of glycogen disease. N = Liver of Neuteboom's operated rabbit (see text)

of normal human livers obtained 2½ hours after death and no such figures have been published in the literature, we entirely depend upon the higher experimental animals for normal controls of postmortem hepatic glycogenolysis. Two experiments carried out in normal rats and one in a glucose-fattened rabbit are recorded in Table I and Figure 2. Moreover, experiments made in cats (14), in a goat and a macacus rhesus (13), and in guinea pigs and rabbits (12b) are presented in Figure 2.⁸ In comparison with these animal experiments, postmortem glycogenolysis in human hyperinsulinism, as observed at 37° C, proceeded at a similar rate in Case 1 and apparently somewhat slower in Case 2, at ice box temperature it held a middle course between the various animals' curves.

By mechanical trauma, such as slicing of the tissue, postmortem glycogenolysis was accelerated in

⁸ Phosphate ions increase hepatic glycogenolysis (Figure 2, D, F)

the 2 clinical cases. This corresponds to similar observations in animal experiments, including the writer's own. The same is true of the increase in Case 1 of postmortem hepatic glycogenolysis by adding highly concentrated bile salts to the suspended liver slices. The proportion of lyo- and desmo-glycogen in the 2 human livers was also approximately the same as in animal controls.

As is shown in the experiment made in the glucose-fattened rabbit (Table I, Figure 2), high liver-glycogen content does not in itself inhibit postmortem glycogenolysis. This was demonstrated earlier by Kimmelstiel (15) in a dog put on "glycogen mast" whose liver glycogen disappeared completely at ice box temperature within 5 days after death. Less active, though distinct glycogenolysis was found by Popper and Wozasek (12b) in rabbits' livers rich in glycogen which were placed in the ice box either directly after death or following a 4-hour incubation at 37° C.

fasting ketonuria, high blood glycogen, hypercholesterolemia, and increased basal metabolism (30b).

From the observations presented in this paper we arrive at the conclusion that pancreatogenic (as well as therapeutic) hyperinsulinism is not identical with typical glycogen disease and, consequently, that typical glycogen disease cannot possibly originate from pancreatogenic hyperinsulinism alone.

This does not mean to say that a certain degree of pancreatogenic hyperinsulinism in conjunction with other endocrine disturbances could not play a part in the pathogenesis of glycogen disease. There are, for instance, 3 autopsy reports in the literature concerning partial enlargement and abundance of the Langerhans islets in von Gierke's Disease ((15, 31) and Krakower's Case 1 (32)). Familial relations to diabetes mellitus are mentioned in 3 cases ((21), Harnapp's Case 2 (30a), Ellis Case 1 (33)). Furthermore, the opinion has been offered that the insulin treated cases of puerile diabetes resulting in hepatomegaly, obesity, and retardation of growth may represent a transition from diabetes into secondary glycogen disease (34). Conversely, Parnas and Wagner's (3) case of glycogen disease turned into diabetes mellitus at the age of 16. On the other hand, the patient of Worster-Drought's (4b) appeared to be almost completely cured after puberty. These puberty changes occurring in glycogen disease, put together with the clinical and pathological reports of insular, thyrogenic, hypophyseal and adrenal⁴ disturbances seem to point to a pluriendocrine disbalance with an overfunction of the insulin apparatus as to the most probable pathogenesis of glycogen disease.

Finally, the question arises whether glycogen disease may not be an example of neurogenic hyperinsulinism. This opinion has lately been advanced by Neuteboom (35). He distinguished 2 "pure types of primary hepatomegaly glyco-genica." The first type is represented by typical glycogen disease of the von Gierke van Creveld description, consisting of a hypofunction of the

injured nerve tissue" (sympathetic pathways) with consequent "relative hyperinsulinism" and "insufficiency of the contrainsular system." For this type, no satisfactory experimental evidence was provided by Neuteboom. The observations made in Case 2 of this report seem to furnish arguments against the supposed identity of glycogen disease with this type of neurogenic hyperinsulinism. In sharp contrast to the first type, the second type of so-called glycogen disease consists of a "hyperfunction of the injured nerve tissue" with a consequent "real reactive hyperinsulinism." For this type, Neuteboom has offered clinical and experimental examples. Clinically, he presented a boy of 13 who early in life had twice suffered undefined injuries. Later, the liver became moderately enlarged. On the mere basis of a histological examination of a liver biopsy the diagnosis of glycogen disease was made (no glycogenolysis test!). Experimentally, spinal injuries were induced on a young rabbit by introducing platinum plates between the left I and II and the right V and VI thoracic segments. During life, the rabbit presented a picture in many respects similar to that of the boy, the liver and heart were enlarged and the blood glycogen was high. However such fundamental symptoms as hypoglycemia, ketonuria, hypercholesterolemia, missing adrenalin response, and insulin sensitivity were absent or reversed in both the boy and the rabbit. After death, the animal's liver and heart were 30 and 40 per cent oversize respectively. Liver glycogen examined 5 hours after death was 5.99 grams per cent (control rabbit 2.25) the heart was not rich in glycogen. Postmortem hepatic glycogenolysis estimated only from 5 to 26 hours after death, was about as active as in our patient, Case 2, with possible neurogenic hyperinsulinism (Table I Figure 1). Most interesting as these observations may be, they apparently do not justify the identification of either type of neurogenic hyperinsulinism with typical von Gierke's glycogen disease.

SUMMARY

In 2 adult cases of spontaneous hypoglycemia, the one probably due to pancreatogenic hyperinsulinism (Case 1 carcinoma of the Langerhans islets with liver metastases) the other possibly due

⁴In *in vitro* experiments with cortical extract, the writer recently found a marked inhibition (up to 80 per cent) of the glycogenolysis of surviving rat liver slices. (To be published in "Endocrinology" 1939)

(3) High liver-glycogen content does not lead by itself to such an inhibition of hepatic glycogenolysis as to produce severe seizures of spontaneous hypoglycemia

(4) Postmortem hepatic glycogenolysis was proceeding at an almost normal or only slightly retarded rate, the glycogenolytic enzyme was therefore active on the livers' own glycogen

(5) Reactions of postmortem hepatic glycogenolysis to varying temperature, mechanical insult and high bile-salt concentration were also normal

(6) Clinically, nevertheless, liver and muscle glycogen depots were mobilized with difficulty through the normal stimuli such as severe hypoglycemia, adrenalin injections and, in Case 2, possibly also ether

These observations made in 2 cases of hyperinsulinism are in good agreement with what is known of insulin physiology. According to this knowledge, one essential action of insulin on the living tissue, especially liver and muscle, consists of an inhibition of the glycogenolytic enzyme resulting in accumulation and fixation of glycogen. In animal experiments, various insulin doses, when added *in vitro*, were able to inhibit postmortem glycogenolysis of normal liver slices (9b) and muscle extracts (18). In the postmortem specimens of the 2 patients' livers such inhibition was present only to a limited degree. Otherwise, in human pathology, only modest inhibitions of postmortem hepatic glycogenolysis have also been observed in insulin-treated cases of infantile alimentary intoxication (Figure 1, *P*), of diabetes mellitus, and schizophrenia (12b, c). In diabetic children injected with insulin for several months or years, a syndrome of enlarged abdomen, hepatomegaly, obesity, and retardation of growth has been described by Mauriac (19) and other French writers (8b).

If the clinical and chemical pathology of spontaneous hypoglycemia due to pancreatogenic hyperinsulinism is compared with von Gierke's glycogen disease, there are a number of striking similarities to be observed in the two pictures. Severe spontaneous hypoglycemia, glycogen accumulation in liver and muscles, and lack of blood sugar response to adrenalin injection are the chief among them. A minor common feature seems to be the stimulation of hepatic glycogenolysis by high bile-

salt concentrations such as has been demonstrated *in vitro* in Case 1 (*cf* Seckel, 9a) and may also occur *in vivo* in glycogen disease when jaundice coincides with the disorder (20, 21, 22), or bile acids are given by mouth to such children (23).

On the other hand, there are outstanding differences between the two diseases. First of all, liver glycogen accumulation is more spectacular in glycogen disease than in hyperinsulinism leading, as it does, to a tumorous enlargement of the organ. Furthermore, organs other than the liver are often involved in glycogen disease, *eg* heart and kidneys. However, accumulation of glycogen as such is by no means pathognomonic of glycogen disease. Similar or even larger amounts of available glycogen may be stored in the liver—to a far lesser degree in other organs—as a result of simple dietary measures (18 to 22.4 grams per cent in dogs (11, 16, 24), *cf* rabbits, Figure 2) or continuous intravenous glucose infusions (upper limit in dogs 22 grams per cent (25)). The fundamental pathology of glycogen disease is rather the almost complete inhibition of postmortem glycogenolysis of the organs affected. For a week or longer, practically no glycogen disappears from the organs, both at 37° C in an intact state (8, 26) and at ice box temperature in an intact as well as a pulped state (27, 28, Table 1, Figure 1). Only in Hertz' (28) case of a young baby, heart and liver showed a rather active postmortem glycogenolysis when pulped and suspended in a phosphate buffer at pH 6.9 and 37° C for 1 or 2 days. The active postmortem glycogenolysis in the intact liver and muscles of Karlstroem's (29) baby (Case 1) indicates, along with an incomplete chemical picture, the presence of either an undeveloped stage or an atypical variety of glycogen disease (*cf* Karlstroem's Case 2). In typical, fully-developed cases of glycogen disease, under natural circumstances, the glycogenolytic enzyme does not act after death on the tissues' own glycogen. This fundamental characteristic of glycogen disease has been shown to be missing in pancreatogenic hyperinsulinism. Here, certainly, we are dealing with the main distinguishing feature between the two diseases. Other specific symptoms of glycogen disease are the absence or rareness of hypoglycemic seizures, high insulin sensitivity, constant

30. Harnapp G O., (a) Zur Klinik der Hepatomegalien mit Kohlehydratstoffwechselstörungen. I Glykogenspeicherkkrankheit. *Monatsschr f Kinderh.*, 1936 66, 169, (b) Idem. III Differential diagnose und Pathogenese der Glykogenspeicherkkrankheit. *Ibid.*, 1936, 66, 194
- 31 Esser, M., and Scheidegger, S., Glykogenkrankheit. Beobachtung eines Falles. *Schweiz. Med. Wehnschr.*, 1937 18, 970
32. Krakower C., The lipid factor in glycogen storage disease. *J Pediat.* 1936 9 728.
- 33 Ellis R. W B., Extreme hepatomegaly in an infant. *Proc. Roy Soc. Med.*, 1933 27, 118
- 34 Gjunc, A., cited by Creveld (8b)
- 35 Neuteboom, J J (a) Bijdrage tot de kennis der hepatomegalia glycogenica. W D Meinema, The sis Utrecht, Holland, 1937 (b) Zur Kenntnis der Glykogenkrankheit. *Klin Wehnschr.*, 1938 17, 1437

to neurogenic hyperinsulinism (Case 2 massive fibroma on right top of the liver), there has been demonstrated a comparatively high liver and muscle glycogen content and an approximately normal or only slightly decreased postmortem hepatic glycogenolysis

Since typical cases of glycogen disease are characterized by an abundance of glycogen accumulated in the liver and other organs and an almost complete inhibition of postmortem glycogenolysis in those organs, neither form of hyperinsulinism is identical with typical glycogen disease and, consequently, typical glycogen disease cannot originate from either form of hyperinsulinism

BIBLIOGRAPHY

- 1 von Gierke, E., Hepato-Nephromegalia Glycogenica (Glykogenspeicherkrankheit der Leber und Nieren) Beitr z. path Anat. u. z. allg Path, 1929, 82, 497
- 2 Wilder, R. M., Allan, F. N., Power, M. H., and Robertson, H. E., Carcinoma of islands of pancreas Hyperinsulinism and hypoglycemia J A M A 1927, 89, 348
- 3 Parnas, J. K., and Wagner, R., (a) Beobachtungen über Zuckerneubildung Biochem Ztschr, 1922, 127, 55, (b) Ueber eine eigenartige Störung des Kohlehydratstoffwechsels und ihre Beziehungen zum Diabetes mellitus Ztschr f d. ges exper Med., 1921, 25, 361
- 4 Worster-Drought, C., (a) Case of enlarged liver with persistent acetonuria and diaceturia Proc Roy Soc. Med., Sect. Dis Child., 1923, 16, 56, (b) Hepatomegaly with persistent ketonuria. Ibid., 1935, 28, 829
- 5 Snapper, I., and van Creveld, S., Un cas d'hypoglycémie avec acétonémie chez un enfant. Bull. et mem Soc. med. d. hop de Paris, 1928, 52, 1315
- 6 Good, C. A., Kramer, H., and Somogyi, M., Determination of glycogen J Biol. Chem., 1933, 100, 485
- 7 Somogyi, M., Sugar determination. J Biol. Chem., 1926, 70, 599
- 8 van Creveld, S., (a) Investigations on glycogen disease. Arch. Dis Childhood, 1934, 9, 9, (b) Glycogen disease. Medicine, 1939, 18, 1
- 9 Seckel, H. P. G. (a) The influence of various physiological substances on the glycogenolysis of surviving rat liver methods, influence of the bile salts Endocrinology, 1938, 23, 751, (b) Idem Influence of insulin added *in vitro* Ibid., 1938, 23, 760
- 10 Willstätter, R., and Rohdewald, M., Ueber den Zustand des Glykogens in der Leber, im Muskel und in Leukocyten (zur Kenntnis der Proteinbindung phys iologisch wichtiger Stoffe) Ztschr f physiol. Chem., 1934, 225, 103
- 11 Schönen, H., Untersuchungen über den Einfluss der Art und Menge der Nahrung auf die Organzusammensetzung und das Stoffwechselgeschehen in verschiedenen Altersstufen Arch f d. ges Physiol, 1932, 230, 179
- 12 Popper, H., and Wozasek, O., (a) Zur Kenntnis des Glykogengehaltes der Leichenleber Wien. Med. Wchnschr, 1929, 79, 456, (b) Idem Ztschr f d. ges exper Med., 1932, 83, 682, (c) Ueber Diastaseschmung in der Leber bei tödlich verlaufender Insulin-Hypoglykämie Virchows Arch. f path Anat., 1933, 288, 673
- 13 Burghard, E., and Paffrath, H., Untersuchungen über den Glykogengehalt der Leber, kritische Untersuchungen über die Methodik der Glycogen- und Kohlehydratbestimmung der Leber Ztschr f Kinderh., 1927, 45, 68
- 14 Evans, C. L., Tsai, C., and Young, F. G., Behaviour of liver glycogen in experimental animals, meth-ods effect of ether and amytal J Physiol, 1931, 73, 67
- 15 Kimmelstiel, P., Ueber Glykogenose. Beitr. z. path Anat. u. z. allg Path, 1933, 91, 1
- 16 Junkersdorf, P., Glykogenspeicherung und Glykogen-speicherungskrankheit. Klin Wchnschr, 1933, 12, 899
- 17 Macleod, J. J. R., The Fuel of Life Princeton University Press, Princeton, 1928, p 50
- 18 Lehmann, H., Action of insulin in cell-free extracts Nature, 1938, 141, 690
- 19 Mauriac, P., Hépatomégales de l'enfance avec troubles de la croissance et du métabolisme des glucides Paris Med, 1934, 2, 525
- 20 Warner, E. C., Case of hepatomegaly due to von Gierke's disease. Lancet, 1933, 1, 1070
- 21 Sundal, A., Glycogenosis (von Gierke's Krankheit) Acta Paediat, 1936, 19, 80
- 22 Anderson, P. M., Glycogen accumulation disease Med J Austral, 1935, 22 (1), 362
- 23 Linneweh, F., Zur Pathogenese der Glykogenkrankheit. Monatsschr f Kinderh., 1937, 70, 238
- 24 Schöndorff, B., Ueber den Maximalwerth des Gesamtglykogengehalts von Hunden. Arch. f d. ges Physiol, 1903, 99, 191
- 25 Butsch, W. L., Glucose tolerance and glycogen storage capacity of dog Am. J Physiol., 1934, 108, 639
- 26 Schönheimer, R., Ueber eine eigenartige Störung des Kohlehydratstoffwechsels Ztschr f physiol Chem., 1929, 182, 148
- 27 Unshelm, E., Die Glykogenkrankheit (Zugleich ein Beitrag zur Frage des hepatogenen Infantilismus) Jahrb f Kinderh., 1932, 137, 257
- 28 Hertz, W., Untersuchungen über den vitalen und postmortalen Kohlehydratstoffwechsel bei Glykogenose und gestörter Schilddrüsentätigkeit. Ztschr f Kinderh., 1936, 58, 259
- 29 Karlstroem, F., Glycogenosis Acta Paediat, 1938, 20, 497

THE FRACTIONATION OF THE IODINE OF THE BLOOD IN THYROID DISEASE

By H J PERKIN AND LEWIS M. HURXTHAL

(From the Research Foundation and the Department of Internal Medicine The Lahey Clinic Boston)

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Investigations concerning the level of iodine in the blood have been an aid in the better understanding of the metabolism of iodine in normal individuals and in patients with goiter. Experimental studies have established a relation between the thyroid gland and the metabolism of iodine. Clinical observations have shown that the abnormal physiological processes manifest in exophthalmic goiter may be alleviated by effecting transitory involution of the hyperplastic thyroid tissue with iodine medication and subtotal resection of the thyroid gland. It is generally agreed that clinical hyperthyroidism is associated with the release of abnormal amounts of iodine from the thyroid. In this connection, the affinity of the thyroid gland for iodine, together with the capacity of thyroid tissue to synthesize organic iodine compounds, plays an important role. Based on the foregoing, the quantitative estimation of the organic iodine in blood should be a closer index of the amount of thyroid secretion than assumptions based on total blood iodine analyses. The purpose of the present communication is to report the results of a study of the fractionation of the iodine in the blood in cases of nontoxic goiter exophthalmic goiter and primary myxedema.

The quantitative estimation of the iodine in the blood is not a simple procedure. The additional technique of fractionating the blood iodine adds greater complexity. A satisfactory method of fractionation should be accurate to the degree that the sum of the iodine analyses of the two fractions should equal the iodine analysis of an aliquot amount of untreated whole blood. In addition, it is important to know approximately the chemical nature of the iodine recovered in each of the fractions. The limits of the method of blood iodine fractionation can best be estimated by adding specific forms of iodine to blood and determining in which fraction the iodine is recovered.

In this present study an alcohol precipitation

method was used for fractionating the iodine of the blood. The procedure was as follows: 20 cc. of blood is secured by venous puncture, 10 cc. of this blood is placed in a clean dry test tube for total iodine analysis, the remaining 10 cc. of blood is placed in a test tube containing a sufficient amount of iodine free sodium oxalate to prevent clotting. The sample of oxalated blood is added directly to 10 volumes of ethyl alcohol. The precipitate is separated from the alcohol blood mixture by filtering through a Whatman (Number 5) filter paper. The residue in the filter paper is washed 3 times with alcohol, using 15 cc. each time. The filter paper containing the precipitate (the so-called organic iodine fraction) is placed directly in a nickel crucible for iodine analysis. (1) The filtrate (the so-called inorganic iodine fraction) is evaporated to dryness and transferred to a nickel crucible for iodine analysis. By exercising care to avoid contamination and loss of iodine (2) in the above procedure, the sum of iodine analyses of the 2 fractions should equal the iodine analysis of the sample of untreated blood.

The adequacy of the above method in fractionating the iodine of the blood was determined in the following manner. A supply of oxalated beef blood was secured from the abattoir. Six samples of 10 cc. each of this blood were used for total blood iodine analysis. To an additional 5 samples (10 cc. each), 38 micrograms of iodine in an organic form were added and total iodine analysis was carried out. These estimations constituted the control results for the fractional analyses. Five samples (10 cc. each) were fractionated according to the procedure outlined above. To an additional 5 samples, 38 micrograms of iodine in an organic form were added and, after mixing, these bloods were fractionated for their iodine content. To another 5 samples of 10 cc. each of blood, 25 micrograms of iodine as inorganic

iodine was nearly twice that of the nontoxic goiter group, being 10.0 micrograms per cent. There appeared to be no correlation between the increase in the organic blood iodine and the elevation in metabolism in individual cases. The proportion of organic to inorganic iodine in the toxic goiter group was approximately the same as that found in the nontoxic goiter group. In the exophthalmic goiter group which had received iodine the organic blood iodine was 8.1 micrograms per cent. This value was less than that found in the exophthalmic goiter group not receiving iodine. In individual cases the decrease in organic blood iodine was not found to be proportional to the decrease in metabolism or the clinical improvement. The total blood iodine of the toxic goiter group receiving iodine was considerably elevated but the additional iodine was present in an inorganic form. In the nontoxic goiter group in which iodine was given, the organic iodine in the blood was greater than that of the nontoxic goiter cases in which iodine was not given. There appeared to be a similarity in the results of the toxic goiter group and the nontoxic goiter group, both of whom had received iodine. The average organic blood iodine of the myxedema cases was 2.5 micrograms per cent. This value was only one-half that of the nontoxic goiter group and one-quarter that of the cases of exophthalmic goiter. The inorganic blood iodine in the myxedema group was proportionately greater than in the other groups not receiving iodine. The results are shown graphically in the accompanying chart.

Attention is now directed to the interpretation of the above results. It is conceded that thyroid tissue has a particular affinity for iodine. In this connection, iodine absorbed from the intestinal tract, chiefly in an inorganic form, is retained and synthesized into specific iodine compounds within the thyroid gland. These iodine products secrete into the circulatory system presumably influence the rate of metabolism. Evidence favoring such a view lies in the fact that inorganic iodine medication (potassium iodide and Lugol's solution) is ineffective in increasing the oxygen consumption of athyroid individuals or of those in whom the thyroid gland has atrophied. In keeping with this theory the organic blood iodine level in the above group of myxedema cases was low; the relative increase in the inorganic blood

iodine of these cases suggests an inability of the thyroid gland to synthesize iodine into an organic form. It is interesting to note that reports in the literature have indicated that the total blood iodine is low in cases of myxedema (3, 4). This is not, however, of diagnostic significance due to overlapping with the range of that found in normals. Estimation of the organic blood iodine may prove to be of greater value in the diagnosis of thyroid insufficiency.

In cases of exophthalmic goiter, an association would appear to exist between the loss of colloid and iodine from the thyroid gland and the increase in the blood iodine level and urinary excretion of iodine. This correlation has been offered as evidence that a true hyperthyroidism exists in cases of exophthalmic goiter (5). If the increase in blood iodine derives from the thyroid gland, there should be a relative increase in the organic iodine in the blood. The present results lend themselves to such a view since the organic blood iodine in the cases of exophthalmic goiter was twice that found in cases of nontoxic goiter and 4 times that present in cases of myxedema. Since no correlation was evident between the level of organic blood iodine and the basal metabolic rate, one should bear in mind the possibility that the increase in metabolism may be associated with the liberation or synthesis of organic iodine compounds not necessarily formed within the thyroid gland. Mention should also be made of the fact that these results are the average values based on a group study and are not necessarily applicable to individual cases. The presence of an elevated or normal organic blood iodine was related to the duration of symptoms in these cases of exophthalmic goiter. The relationship has previously been established for the total blood iodine (6).

A regression of the signs and symptoms in patients with hyperthyroidism on iodine treatment (that is 10 minims Lugol's solution 3 times daily) is an established fact. The clinical improvement is associated with an involution of the acinar elements of the thyroid gland. This histological change has been regarded as evidence of a reduction in the secretory capacity of the thyroid. In connection with this view Holst, Lunde, Closs and Pederson (7) and Lunde, Closs and Pederson (8) reported that iodine medication in cases

iodide were added and, after mixing, the bloods were fractionated. The results of analysis of the above procedures are shown in Table I.

TABLE I

	Average total blood iodine	Average organic blood iodine	Average inorganic blood iodine
	micrograms	micrograms	micrograms
10 cc. of blood	0.85		
10 cc. of blood + 3.8 micrograms of organic iodine*	4.62		
10 cc. of blood		0.82	0.12
10 cc. of blood + 3.8 micrograms of organic iodine*		4.46	0.24
10 cc. of blood + 2.5 micrograms of inorganic iodine†		0.84	2.65

* Thyroid protease was used.

† Potassium iodide was used.

As will be seen from Table I, the average total blood iodine analysis was 0.85 micrograms per 10 cc. The actual error was ± 0.12 micrograms. The recovery of iodine was satisfactory in the samples of blood to which 3.8 micrograms of organic iodine were added. In the fractionation of the whole blood, the organic iodine result was 0.82 micrograms per 10 cc. and the inorganic iodine, 0.12 micrograms. Although the sum of these 2 fractions exceeds the total iodine analysis of 0.85 micrograms, the results are still within the limits of actual error. In the fractionation of the blood samples to which the organic iodine compound was added, the recovery of iodine was almost complete in the organic blood iodine fraction. The slight increase in the inorganic fraction (0.24 micrograms) was attributed to a small amount of inorganic iodine present in the thyroid protease. In the fractionation of the blood samples to which the inorganic iodine was added, the additional iodine was quantitatively recovered in the inorganic blood iodine fraction.

The organic iodine compound used in the above experiments was a protease made from human thyroid tissue by Dr W. T. Salter, Harvard Medical School, Boston, Massachusetts. This thyroid protease has a molecular weight of approximately 7000. Thyroxine (molecular weight of 777), when placed in solution and added directly to blood, cannot be completely recovered in the organic fraction by the above methods. Thus it would appear that the alcohol precipitation method

used in fractionating the iodine of the blood was only capable of recovering in the so-called organic fraction iodine compounds with a molecular weight of 7000 and greater. The foregoing data are considered to establish the accuracy and the limitations of the method used in the present study of the fractionation of the iodine in the blood.

Fractional analysis of the blood iodine has been carried out on 218 individual patients. The cases have been divided into groups dependent upon the degree of thyroid activity as clinically estimated. The cases included 65 patients with nontoxic adenomatous goiter (normal), 98 patients with exophthalmic goiter who had never received any iodine medication, 38 patients with exophthalmic goiter who had received iodine for some time (at least a month) up to 24 hours prior to the blood iodine analysis, 7 patients with nontoxic goiter who had likewise received iodine, and 10 patients with untreated primary myxedema. Total blood iodine analyses and fractional blood iodine analyses were carried out in each case. The average blood iodine results of each group of cases are shown in Table II.

TABLE II

	Average total blood iodine	Average organic blood iodine	Average inorganic blood iodine
	micrograms per cent	micrograms per cent	micrograms per cent
Nontoxic goiter (no iodine)	8.0	5.3	3.2
Exophthalmic goiter (no iodine)	16.3	10.0	6.5
Exophthalmic goiter (on iodine)	19.4	8.1	10.8
Nontoxic goiter (on iodine)	17.1	8.7	8.2
Primary myxedema	5.7	2.5	3.1

As will be seen from Table II, of the total blood iodine of 8.0 micrograms per cent of the nontoxic goiter group, 5.3 micrograms of iodine were present as organic iodine.¹ The difference obtained by actual iodine estimation, amounting to 3.2 micrograms per cent, was inorganic iodine. In the group of patients with exophthalmic goiter which had not received iodine, the organic blood

¹ The term organic blood iodine used throughout designates the iodine recovered in the alcohol insoluble fraction (see method).

clinical improvement of the patient. The effect of iodine treatment on the organic blood iodine may well be influenced by the time the blood analysis was done in relation to the last dose of iodine received and to the length of time iodine was administered. Different results with diverse interpretations might have been secured had the blood for analysis been taken at a different time. However, with these reservations in mind, one may state that under certain conditions, iodine medication in cases of exophthalmic goiter results in a decrease in the organic iodine of the blood. Insofar as this organic blood iodine fraction comprises the products of thyroid secretion it would appear that iodine medication in cases of hyperthyroidism decreases the amount of iodine secreted by the thyroid gland.

In patients with nontoxic goiter without signs or symptoms of hyperthyroidism, but who were receiving iodine, the finding of an elevated organic blood iodine is difficult to explain. The results indicate that apparently normal individuals, if given iodine, may show an increase in the organic iodine of the blood. This observation suggests an explanation of the inability of iodine therapy to control completely the signs and symptoms of clinical hyperthyroidism.

SUMMARY

A A method for the fractionation of the iodine of the blood has been described. The limitations of the method have been established.

B Compared with the normal,

1 A decrease in the level of the organic iodine of the blood was found in patients with primary myxedema.

2 Patients with exophthalmic goiter who had not received iodine therapy were found to have

an increase in the level of organic iodine in the blood.

3 A relative decrease in organic blood iodine was found in patients with exophthalmic goiter following iodine medication.

4 Patients with nontoxic goiter receiving iodine showed a relative increase in the organic iodine of the blood.

BIBLIOGRAPHY

- 1 Perkin, H. J., Determination of iodine in blood. *Biochem. J.*, 1933 27 1078.
- 2 Perkin, H. J., and Cattell, R. B., The practicability and significance of blood iodine estimations. *New York State J. Med.*, 1936 36 1033.
- 3 Schittenhelm, A. and Eisler, B., Der Blutjodspiegel in seiner pathologisch physiologischen und klinischen Bedeutung. *Klin. Wchnschr.*, 1932, 11 6.
- 4 Elmer A. W., and Scheps, M., The iodine content of blood and of urine and the basal metabolic rate, their value in the diagnosis of the function of the thyroid gland. *Acta med. Scandinav.*, 1934, 82, 126.
- 5 Curtis, G. M., Davis, C. B., and Phillips, F. J., Significance of the iodine content of human blood. *J. A. M. A.* 1933 101 901.
- 6 Perkin, H. J., and Lahey, F. H., Exophthalmic goiter. Relation between the blood iodine level and the duration of symptoms in 305 cases. *Arch. Int. Med.*, 1938 61, 875.
- 7 Holst, J., Lunde, G., Gloss, K., and Pederson, O. C., Über den Inneren Jodstoffwechsel Bei Primären Thyreotoxikosen (Primär Basedow). *Klin. Wchnschr.*, 1928 7 2287.
- 8 Lunde, G., Gloss, K., and Pederson, O. C., Untersuchungen über den Jodstoffwechsel. *Biochem. Ztschr.*, 1929 206, 261.
- 9 Dodds, E. C., Lawson, W., and Robertson, J. D., Variations in the iodine content of the blood in hyperthyroidism and nontoxic goiter. *Lancet*, 1932, 223, 608.
- 10 Trevorrow, V., Studies on the nature of iodine in blood. *J. Biol. Chem.* 1939 127, 737.

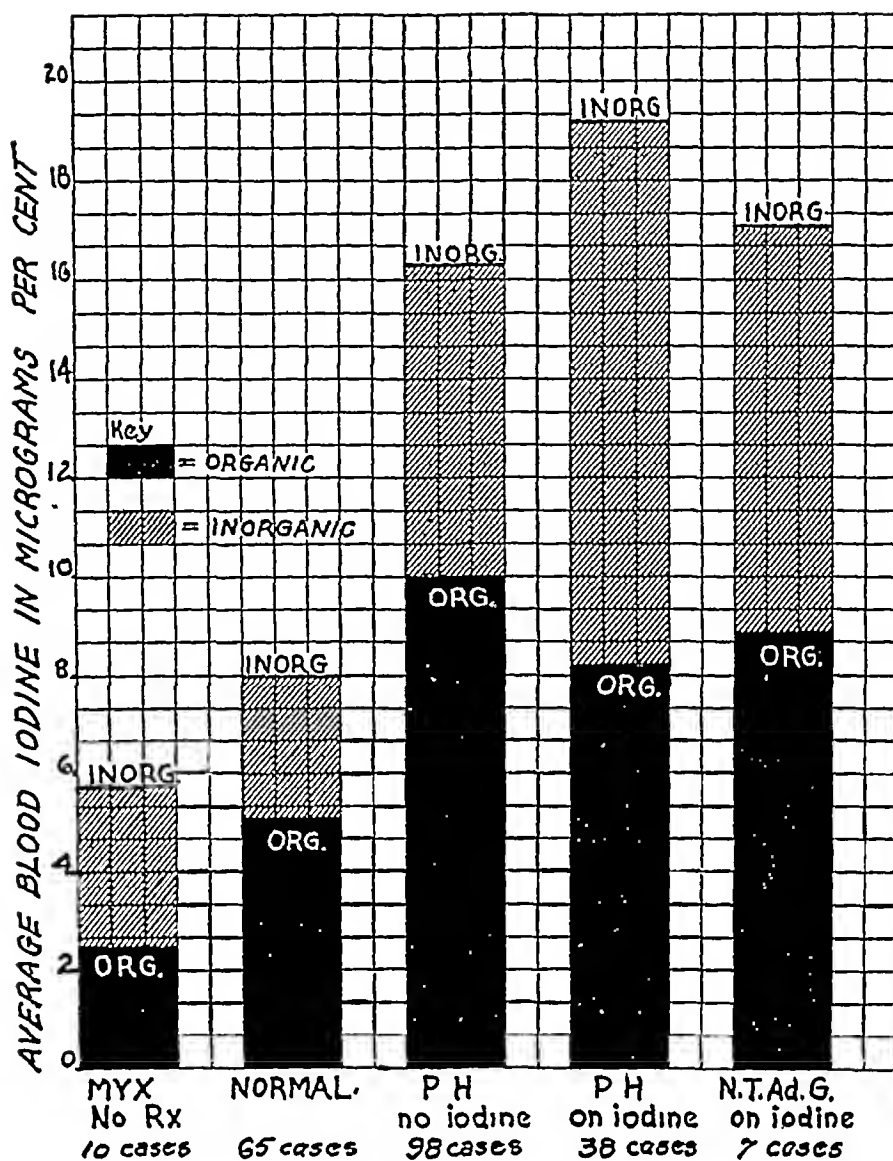


FIG. 1 THE RELATION OF THE ORGANIC AND INORGANIC IODINE OF THE BLOOD TO THE TOTAL BLOOD IODINE

of hyperthyroidism results in a decrease in the organic blood iodine. They presented evidence that the organic blood iodine decreased to normal proportionately with the basal metabolic rate. Using a method of alcohol precipitation followed by Soxhlet extraction, it was their opinion that the organic blood iodine fraction contained the active principle of the thyroid gland. Dodds, Lawson and Robertson (9), using similar methods of study, were unable to confirm these observations. Relative to the aforementioned studies, the recent experimental observations of

Trevorrow (10) on the nature of the iodine in blood are of interest. Our results (see Table II) showed that the average organic blood iodine of patients with exophthalmic goiter receiving iodine was less than in patients not receiving iodine. These results, however, can only be interpreted as a general principle derived from a group study. Individually, exophthalmic goiter cases, not having had iodine, have been noted with a normal or even a subnormal organic blood iodine. Iodine treatment in these cases may result in an increase in the organic blood iodine associated with a

SERUM LIPOIDS AND PROTEINS IN HYPOTHYROIDISM¹

By E. F. GILDEA, E. B. MAN, AND J. P. PETERS

(From the Departments of Psychiatry and of Internal Medicine Yale University School of Medicine New Haven and the Medical Service of the New Haven Hospital)

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Previous investigators (1, 2, 3, 4, 5) have shown that a high serum cholesterol is commonly found in patients presenting the classical symptoms of myxedema. There has been a difference of opinion (6), however, as to whether high cholesterol invariably occurred and as to whether it was a sufficiently fundamental part of the syndrome to be of value in weeding out the atypical forms of hypothyroidism from the complex group of patients with low basal metabolic rates and such symptoms as retardation, weakness, easy fatigability, sensitivity to cold, falling hair, dry skin, obesity, etc. Certain authors, and particularly Hurxthal, have concluded that the level of cholesterol constitutes a valuable index of the degree of hypothyroidism, while others (7, 8, 9, 10) have found it unreliable and not an essential part of the syndrome. A contributing factor to this disagreement has been the difference in methods employed.

The basal metabolic rate has not proved to be an infallible criterion of hypothyroidism. For example, it may be low without the syndrome of sensitivity to cold, edema, dry skin and coarse hair, or the patient may be so tense and excitable that relaxation essential to the measurement of the true basal metabolic rate is impossible.

The present investigation was undertaken to study whether or not the levels of serum cholesterol, fatty acids and phosphatides might furnish accurate criteria of thyroid deficiency and of the value of administering desiccated thyroid. A second purpose was to evaluate the effect of administering thyroid at the height of lipemia. Disorders in protein metabolism have been also described as occurring in myxedema but the apparent changes in serum proteins may well have been compensatory responses to the disturbance in water metabolism. In order to obtain further insight into this problem serum proteins and

albumin and globulin fractions have been also investigated in these patients.

MATERIALS AND METHODS

In order to evaluate the significance of the level of lipoids in myxedema as many as possible of the criteria of hypothyroidism have been utilized in the study of each patient. These criteria have been outlined under the categories of clinical symptoms, basal metabolic rate, height of serum total proteins as contrasted with serum cholesterol and other lipoids and proteins.

The following clinical symptoms have been considered indicative of hypothyroidism. Of first importance was the presence of edema, usually beginning as puffiness about the eyes. Dry skin and coarse dry hair associated with scanty eyebrows, general slowness of motor and mental activities, or more briefly retardation, and slow pulse have been considered significant. Subjective symptoms have as always, proved difficult to evaluate. Sensitiveness to cold, complaints of easy fatigability and general weakness have been particularly considered.

A basal metabolic rate below minus 20 per cent has been accepted as an essential criterion of classical myxedema. It has been recognized, however, that occasionally patients with clinical signs of myxedema that disappear promptly under thyroid therapy may not have such low basal metabolic rates.

Previous work has shown that serum proteins may be elevated in hypothyroidism. Values of 77 per cent (11) or higher have been considered as suggestive of hypothyroidism.

Cholesterol has been accepted as significantly above the normal limits for the method when the level was 300 mgm per cent or more. This high figure has been chosen instead of 250 mgm per cent, the upper limit for the majority of people in good health, because 5 out of 100 of these nor-

¹This work was aided in part by a grant from the Knight Fund, Yale University School of Medicine.

TABLE IV

Patients with low basal metabolic rates with weakness and multiple complaints, not improved by thyroid administration

Number sex and age	Thy roid dose	Edema	Weight	Pulse	Dry skin	Coarse and thick hair	Retardation		Weak ness	Pain and aches in muscles and joints	Sensi- tivity to cold	Basal metab- olism	Serum		Pro- tein
							Physi- cal	Men- tal					Choles- terol		
years	grains per day		kilo- grams									per cent	mgm. per 100 cc.	per cent	
A7885	0	0	65.0	52	+	+	++	+	++	++		-17	300	7.2	
F 55 (T)	2	0	62.8	68	+	+	++	+	++	++		-1	192	6.6	
P1603	0	0	54.7	88	++	+	++	+	++	++	0	-1*	263	7.0	
F 28 (T)	3	0	54.0	80	++	+	+	+	+	++	0	+5*	167	6.5	
3															
F 34	0	0	53.4	56	0	0	++	0	++	++	++	-19	191	6.1	
	2	0	53.0	64	0	0	++	0	++	++	++	-3	146	5.8	
A6147	0	0	60.5	70	+	+	+	+	++	++	++	-10	277	6.4	
F 66	1½	0	60.5	72	+	+	+	+	++	++	++	+9	213	6.1	
58434	0	0	79.5	76	0	0	+	+	+++	+++	0	-15	175	7.2	
F 44	1½	0	79.7	66	0	0	+	+	+++	+++	0	-1	137	7.2	
A56396	0	0	57.3	60	0	0	++	0	++	++	++	-14	200	6.7	
F 40	2	0	56.8	64	0	0	++	0	++	++	+	-6	189	6.7	

(T) Previous thyroidectomy

* Unsatisfactory

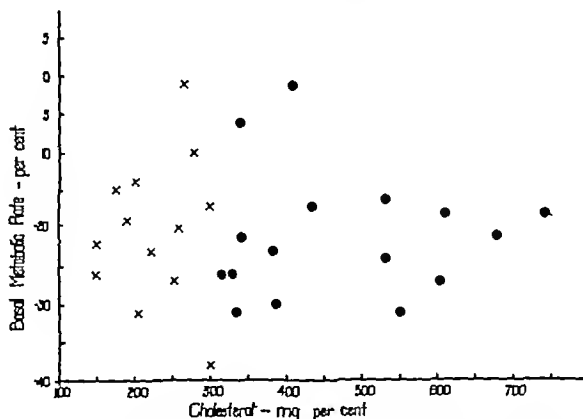


FIG. 1 BASAL METABOLIC RATE AND SERUM CHOLESTEROL AS INDICATORS OF SUBSEQUENT THYROID THERAPY

Circles indicate values in patients before treatment who improved on thyroid therapy

Crosses indicate values in patients before treatment who did not improve on thyroid therapy

metabolic rates. Two were moderately obese (A6147, 58434). Two had had previous thyroidectomies. Their initial basals, with the exception of P1603, ranged from minus 10 to minus 19 per cent. They experienced no, or only slight,

subjective improvement with thyroid administration.

The following figures have been employed to demonstrate relationships which could not be readily illustrated in tables. Figure 1 shows the

Only 5, 54183, P1536, A78256, 1, and 85071 had basal metabolic rates below minus 20 per cent. Patient A39091 had hypertensive cardiovascular disease with decompensation, thereby making the results of basal metabolic rate determinations of uncertain clinical significance. Furthermore, on account of the cardiac condition the thyroid therapy was not pushed to the point of complete disappearance of symptoms attributable to hypothyroidism. Patient A67142 had had an acute nephritis following picric acid treatment of severe sunburn at about the time symptoms of hyperthyroidism were noted. The nephritis cleared up partially and he did well after thyroidectomy until 3 months later when the symptoms of myxedema, noted in Table II, appeared. At this time some of the edema and weakness might have been due to the chronic nephritis which persisted. Patient 1536 also had a mild chronic nephritis probably secondary to a former chronic pyelitis. All of the 11 patients in this group were relieved of most of the symptoms attributed to hypothyroidism by $\frac{1}{2}$ to $2\frac{1}{2}$ grains of thyroid daily. One, 85071, was extremely slow in recovering on thy-

roid therapy. This patient, a woman, was a poor informant of low intelligence who also had mild diabetes. Such complaints as weakness, pains and aches, mental and physical retardation, were difficult to evaluate. She obtained some relief on thyroid therapy. A subsequent course on placebos gave similar results. More recently, however, a longer period of thyroid therapy resulted in more convincing evidence of increasing strength, motor speed, and general improvement.

Group 3, Table III, includes patients with only some symptoms suggesting myxedema, but with basal metabolic rates below minus 20 per cent. The analysis of the symptoms reveals, however, that none had edema, that changes in the skin were absent or moderate in degree, and that only one complained of sensitivity to cold. The administration of thyroid produced little improvement in these patients, even when the daily dose was 4 or 5 grains.

In Table IV are 6 patients who suffered from a miscellaneous variety of symptoms but they all had in common incapacitating weakness and multiple pains and aches. Some had low basal

TABLE III

Patients with basal metabolic rates below minus 20 per cent and a few symptoms suggestive of myxedema, not improved by thyroid administration

Number sex and age	Thy- roid dose	Edema	Weight	Pulse	Dry skin	Coarse and thin hair	Retardation		Weak- ness	Pain and aches in muscles and joints	Sensi- tivity to cold	Basal metab- olism	Serum Choles- terol	Protein
							Physi- cal	Men- tal						
years	grains per day		kilo- grams									per cent	mgm per 100 cc	per cent
A59140	0	0	67.0	54	0	0	+	+	+	++	++	-26	149	6.1
F 27 (T)	2½	0	67.5	70	0	0	0	+	+	++	+	-8	114	5.7
A44014	0	0	83.0	54	+	++	+	+	+	++	0	-31	204	6.7
F 39 (T)	4	0	92.0	64	+	++	+	+	+	++	0	-10	156	6.3
5	0	0	80.0				+	+	++	+++		-20	258	7.0
F 50	5	0	74.0				+	+	++	++		+1	157	6.5
5253	0	0	64.0	60	++	+	+	0	++	+++		-27	252	5.9
F 49	4	0	66.5	86	++	+	+	0	++	+++		-1	171	5.9
P1667	0	0	50.0	75	0	++	++*	++	+++	0	0	-38	300	7.0
F 52	4†	0	47.0	75	0	++	++	++	+++	0	0	+1	119	6.4
2	0	0	97.0	60	+	+	+	0	+	0	0	-23	222	6.9
M 35	2	0	97.0	60	+	+	+	0	+	0	0	-3	180	6.4
6	0	0	72.2		0	0	++	+	+	0	0	-22	149	6.6
M 42	4	0	75.4	74	0	0	+	+	+	0	0	-1	94	5.9

(T) Previous thyroidectomy

* Psychosis with alternating apathy and excitement.

† Thyroxin equivalent to 4 grains thyroid

ing that the concentration of serum cholesterol is related to the activity of thyroid substance in the body. The ultimate conclusion was that the patients in groups I and II had hypothyroidism because the therapeutic responses both as to physical signs and cholesterol effect were marked, although the basal metabolic rates were not always very low before treatment.

The patients in Table III were suspected of having hypothyroidism because the basal metabolic rates were definitely below minus 20 per cent and associated with a number of symptoms of hypothyroidism. They did not respond, however, to treatment with thyroid and in this respect differed from the patients in Tables I and II. A comparison of the 3 tables also demonstrates that

none of the patients in Table III had cholesterol values that were as high as those in I and II. In fact all of the cholesterol values were under 259 mgm. per cent, with the single exception, P1667, whose cholesterol was 300 mgm per cent.

A comparison of the patients in Table IV with those in I and II shows again, although less strikingly, that the presence of a low basal metabolic rate and some of the symptoms attributed to hypothyroidism does not indicate that the symptoms will necessarily be relieved by thyroid administration. It is also apparent that these patients like those in Table III have much lower cholesterol values and also lower proteins.

These data indicate that the effects of administering thyroid can be foretold more accu-

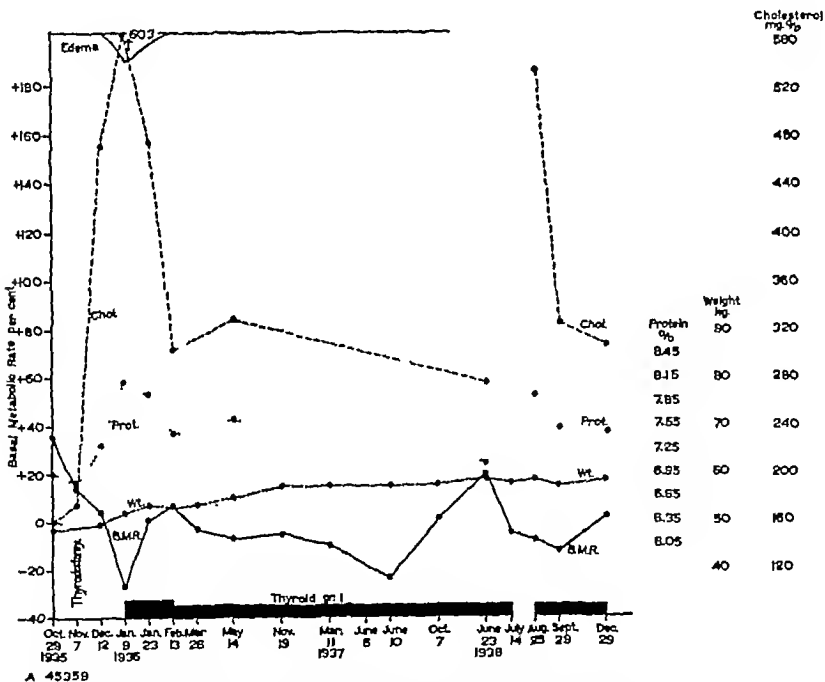


FIG. 5 RELATIVE CHANGES IN BASAL METABOLIC RATE, WEIGHT, EDEMA, SERUM CHOLESTEROL AND PROTEINS OF A45359

Thyroid dosage indicated by solid black block gaps, no thyroid narrow block, grains 1 wide, grains 1½ per day

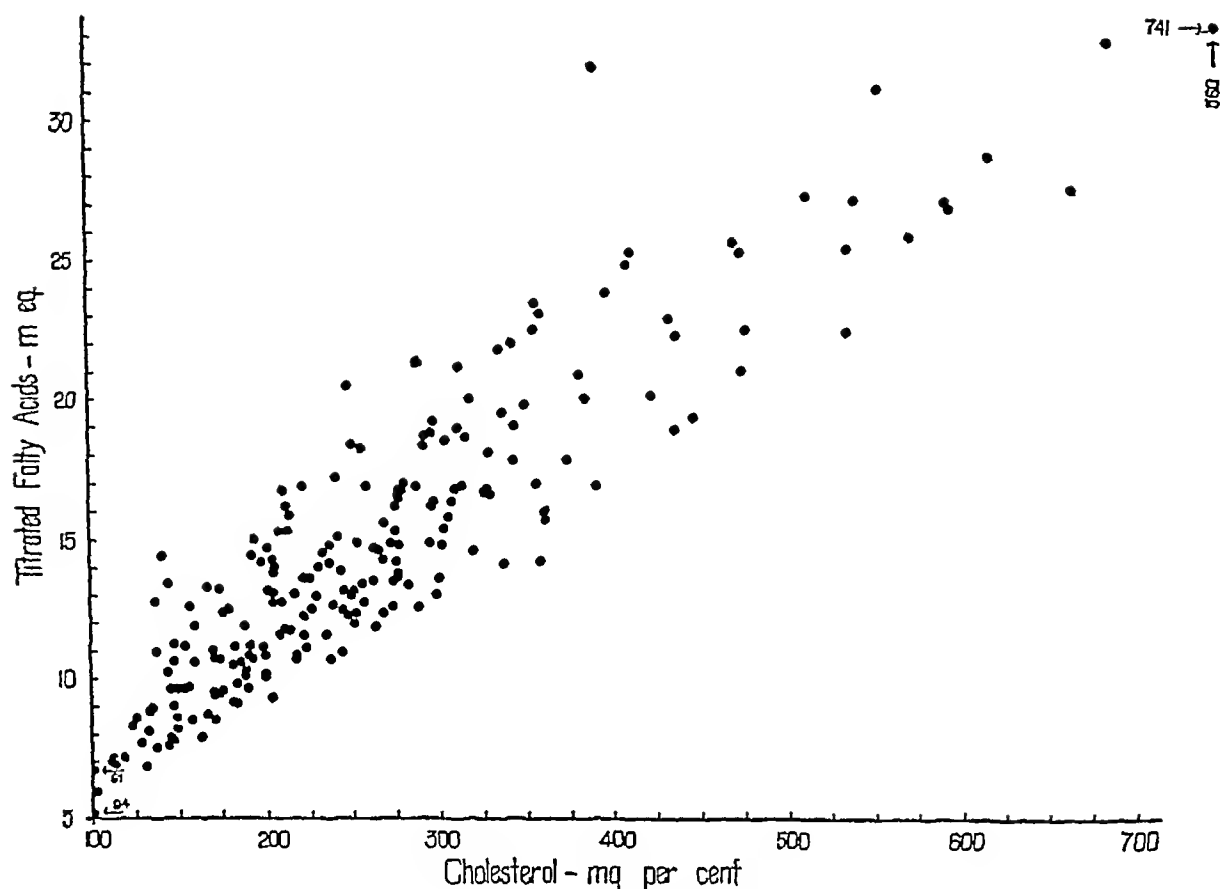


FIG 4 COMPARISONS OF SERUM FATTY ACIDS AND CHOLESTEROL IN 214 STUDIES ON 29 PATIENTS

in 2 While these changes were taking place the serum cholesterols fell from the abnormally high initial concentrations ranging from 335 to 603 mgm per cent to the normal range of 275 mgm per cent or less, the lowest being 176 mgm per cent

The patients included in Table II were relieved by thyroid of the symptoms referable to hypothyroidism almost as completely as those in group 1 But before they were tested in this fashion, there were one or more reasons for determining whether or not hypothyroidism was present in each case It can be seen in Table II, for example, that the basal metabolic rates of 6 cases ranged from only minus 18 per cent to minus 1 per cent (While edema was present in 5 patients, there was enough evidence in 3 of chronic nephritis or cardiovascular disease to obscure its significance) Also, the other symptoms such as dry skin, coarse thin hair, retardation,

weakness, and sensitivity to cold, were frequently not marked enough to be helpful in diagnosis It is therefore noteworthy that the initial cholesterols were all abnormally high, in fact their range of 315 to 741 mgm per cent was similar to that of the myxedematous patients in Table I The relief of symptoms and the extent of the fall of cholesterol on treatment with $\frac{1}{2}$ to $2\frac{1}{2}$ grains of thyroid also corresponded closely with the group in Table I, with the exception of cases 54183, A33872 and P1536 In these latter patients the symptoms due to hypothyroidism were relieved and their cholesterols fell more than 250 mgm per cent Yet, owing to complicating conditions, weakness and other symptoms persisted and the cholesterols remained above normal

Omission of the thyroid in 4 of the patients in groups 1 and 2 produced a complete reversion in symptoms and a parallel rise in cholesterol, thereby confirming the previous evidence indicat-

se as was that of the phospholipids. It is interesting that the hypothyroid patients, A58944, A39091, who had fatty acids high in proportion to cholesterol, were those who also had microanemias and some malnutrition. On the other hand, those who had cholesterol particularly high in proportion to the fatty acids, A33872, A42, P1536, 1, and P1667, all had comparatively low proteins, 74 to 61 per cent. A67142, P1536 also had mild forms of chronic nephritis. Furthermore, the fatty acids seemed less responsive to thyroid and in 4 of the non hypothyroid patients failed to fall with the cholesterol levels. The serum proteins of the 5 myxedematous patients in Table I were determined before thyroid treatment and in 3 patients after

omission of thyroid. Of these 8 determinations of serum proteins without recent thyroid therapy, 7 values were between 74 and 84 per cent. These values are distinctly higher than would be expected in 5 normal subjects. According to the data of Peters and Eisenman (11), the serum proteins of normal subjects vary from 5.7 to 8.0 per cent and 90 per cent of the values lie between 6.3 and 7.7 per cent. Only 5 of the 11 patients in group 2 had proteins above 74 per cent. Although improvement from thyroid administration can be predicted from the initial high level of serum proteins in clear-cut cases of myxedema, it cannot be predicted from the proteins in doubtful cases.

The changes which occurred in the proteins of

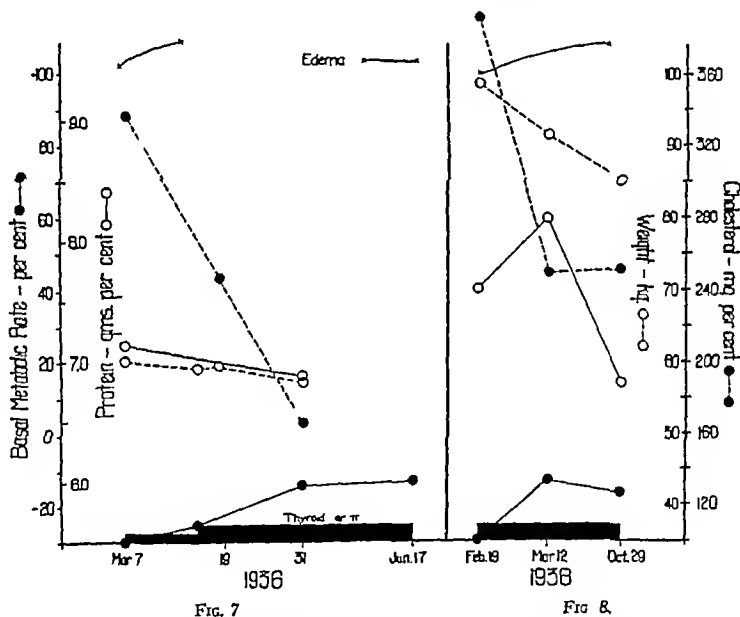


FIG 7. RELATIVE CHANGES IN BASAL METABOLIC RATE, WEIGHT, EDEMA, SERUM CHOLESTEROL AND PROTEINS OF A62475

Thyroid dosage indicated by solid black block, gaps, no thyroid; narrow block, grains 1 wide, grains 2 per day

FIG 8. RELATIVE CHANGES IN BASAL METABOLIC RATE, WEIGHT, EDEMA, SERUM CHOLESTEROL AND PROTEINS OF A58944

Thyroid dosage, grains 2 per day indicated by wide black

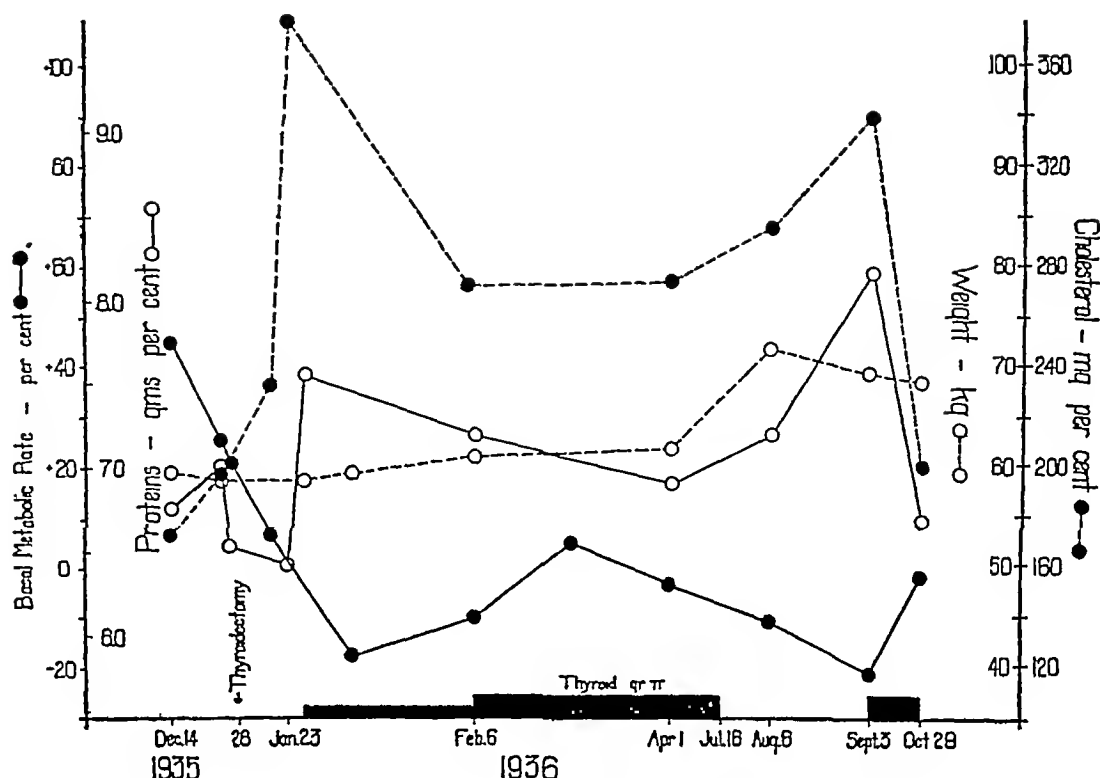


FIG 6 RELATIVE CHANGES IN BASAL METABOLIC RATE, WEIGHT, SERUM CHOLESTEROL AND PROTEINS OF A43137

No edema was observed. Thyroid dosage indicated by solid black block, gaps, no thyroid, narrow block, grains 1, wide, grains 2 per day

rately if, in addition to determination of the basal metabolic rate, the cholesterol is also measured. This point is illustrated in Figure 1 where it can be seen that all of the patients who improved on thyroid, denoted by circles, had initial cholesterol well above 300 mgm per cent, while those who did not, represented by crosses, had far lower amounts with one exception, P1667, who had 300 mgm per cent.

Furthermore, the serum cholesterol proved to be very sensitive to the administration of thyroid. Inspection of Figure 2 shows that the cholesterol fell after thyroid in all of the patients. However, the fall in cholesterol took place on 1 to 2 grains of thyroid in the myxedematous patients (Tables I and II) while much larger amounts were required in the others (Tables III and IV). The actual magnitude of the decreases in cholesterol is dependent to some extent on the initial level of cholesterol. The relation of the height of the cholesterol to the severity of myxedema is

considered later in the discussion. The sensitivity was also apparent when thyroid therapy was omitted and a reversion to the pre-treatment condition occurred. Examples of these responses may be seen in Figures 5, 6 and 9. In a number of patients—for example, A45359, Figure 5, A43137, Figure 6—cholesterol changed earlier than the basal metabolic rate.

Lipoid phosphorus As can be seen in Figure 3, where all of the determinations on the 4 groups of patients have been plotted, there is essentially a straight line relationship between cholesterol and lipoid phosphorus. This correlation is so close over the whole range that, within narrow limits, the concentration of either component could be predicted from analytical measurement of the other.

Fatty acids The fatty acids in most of the patients tended to vary with the cholesterol. But, as can be seen in Figure 4 where they have been plotted against cholesterol, the relation was not as

again resulted in a decrease. Further inspection of the charts reveals that the proteins were not regularly affected as rapidly as were serum cholesterol basal metabolic rate, or symptoms.

In group 2 the serum proteins of A39091, A67142 and P1536 can hardly be included because the first patient suffered from a severe cardiac disorder and the other 2 from low grade chronic nephritis. The serum proteins of 5 of the remaining 8 patients were 0.5 to 1.8 per cent lower after thyroid. Six of the 7 patients in group 3 had a decrease of 0.4 to 0.7 per cent in proteins after thyroid. In group 4, consisting of patients who were given thyroid because of vague symptoms, there is no correlation between serum proteins and thyroid administration.

All the patients in group 1 except A45359 lost weight after the administration of thyroid. In group 2, 5 of the 8 patients without cardiac or renal complications weighed less after thyroid. Two of these, 10670 and 1 had simultaneous diminutions in body weight and in serum proteins. No correlation could be discovered between changes in clinical evidences of edema, including weight loss, and serum proteins.

DISCUSSION

The serum cholesterol of every patient in the 4 tables fell after the administration of thyroid. Some of these patients, notably A44014, 6, 5253 and P1667 required large doses to effect these results. In Tables III and IV (with the exception of P1667 who was injected intravenously with large doses of thyroxin) the decreases in cholesterol in 8 of the 12 patients were between 11 and 65 mgm per cent. It has been shown previously that serum cholesterol in the same individual varies over a wide range if it is quantitated repeatedly (18, 19, 20). In our own experience (18) the cholesterol of a normal male studied at intervals for 2½ years varied from 173 to 239 mgm. per cent, and in a normal female studied 4 years the cholesterol varied from 203 to 257 mgm per cent. The fall in cholesterol in patient 6 from 149 to 94 mgm per cent (Table III) may be of more significance than a similar variation in the cholesterol of the normal male from 239 to 173 mgm per cent. It is preferable, therefore, to express the decrease in cholesterol as the per cent of the highest value for the indi-

vidual. Percentage decreases in cholesterol in 6 of the 12 patients in Tables III and IV (with omission of P1667) were less than the percentage decrease in the cholesterol of the normal male. It is obvious that, though thyroid invariably lowered the serum cholesterol, the actual diminutions in half the patients in Tables III and IV did not exceed the limits of normal variation.

Examination at frequent intervals of the serum of 3 patients in the third and fourth groups has shown that the decrease in cholesterol was only transient. These 3 patients were an obese male (2) with no clinical signs of myxedema, a hypochondriacal obese female (A6147) 66 years old, and a 27 year-old female (A59140) who developed a unilateral exophthalmos after thyroidectomy. Before deciding however that the fall in cholesterol is transient, the size of the dose of thyroid and the amount of increase must be considered. A male patient weighing 182 kilograms showed no reduction in cholesterol after 2 grains of thyroid per day. That the cholesterol might have fallen if the dosage had been increased is suggested by the fact that many of the patients in groups 3 and 4, whose weight did not approach 182 kgm required 2 or more grains of thyroid to reduce the cholesterolemia.

The question as to whether or not the oral administration of thyroid permanently lowers the serum cholesterol of normal as well as of myxedematous individuals, has been attacked from different angles by many investigators. Page and Farr (21) have recently confirmed earlier observers that thyroid ingestion does not decrease the hypercholesterolemia of nephrosis. Blumgart and coauthors (22, 2) have found that, after total ablation of the thyroid for heart disease, serum cholesterol rises. The cholesterolemia of these thyroidectomized patients is usually influenced more markedly by small doses of thyroid than the serum cholesterol of patients without thyroidectomies. Hurvital (3) gave thyroid to 3 'apparently normal persons' and reported a fall in the blood cholesterol of each. However, 2 of the 3 before thyroid had basals of minus 16 and minus 35 per cent. This seems an unfortunate selection of normal subjects for such an experiment. In the third subject thyroid was only given

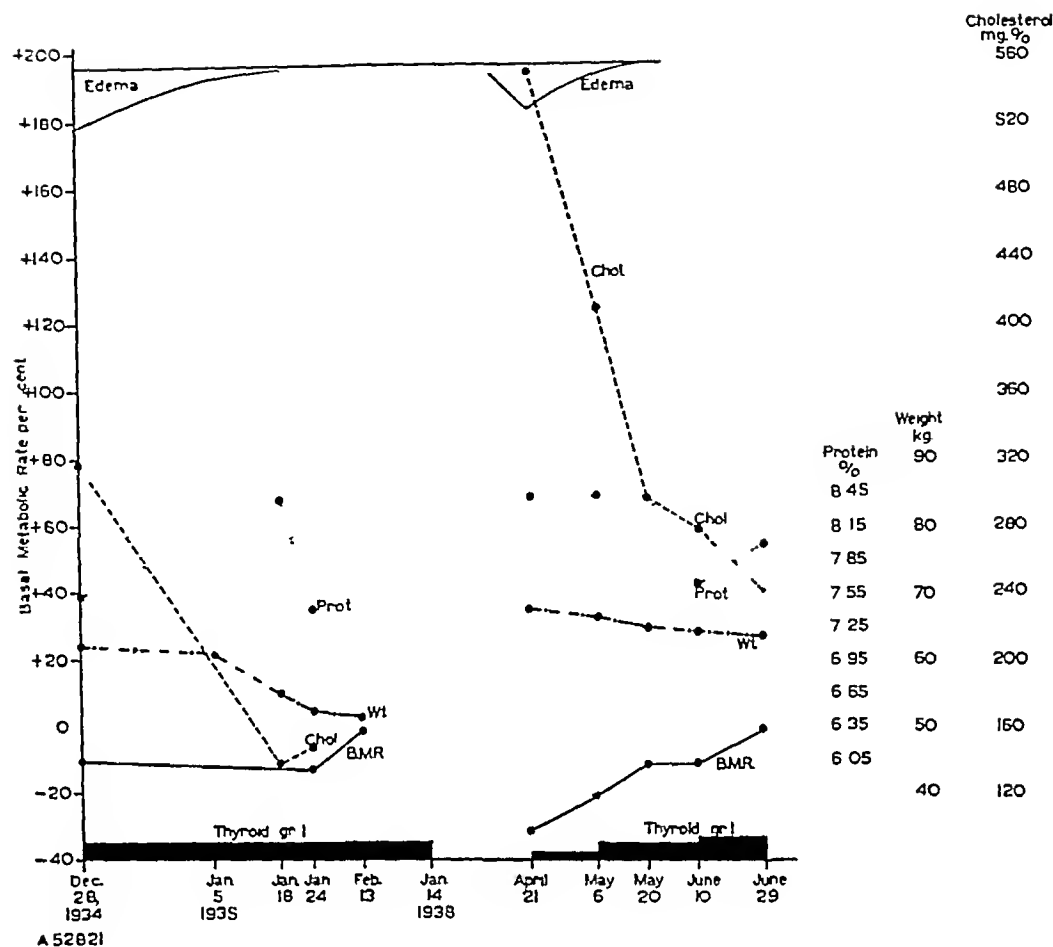


FIG 9 RELATIVE CHANGES IN BASAL METABOLIC RATE, WEIGHT, EDEMA, SERUM CHOLESTEROL AND PROTEINS OF A52821

Thyroid dosage indicated by black block, gaps, no thyroid, narrow block, grains $\frac{1}{2}$, medium, grains 1, wide, grains $1\frac{1}{2}$ per day

the 5 myxedematous patients after treatment are illustrated in Figures 5, 6, 7, 8 and 9. In 3 of the 5 patients in group 1 after thyroid administration the proteins fell from 0.2 to 1.4 per cent. In the fourth patient, A58944 (Figure 8), the proteins rose from 7.6 to 8.2 per cent in the face of clinical improvement manifested by a loss in weight, a marked fall in cholesterol and rise in basal metabolic rate. Eight months later, however, when recovery had taken place, the proteins had dropped to 6.8 per cent. The fifth patient, A52821, was anemic and had been on a deficient diet before thyroid therapy. Thyroid administration and a more adequate diet were followed in 21 days by a weight loss of 7 kgm, a rise in proteins, marked clinical improvement, fall in

lipoids, and rise in basal metabolic rate. Four years later, when the patient felt so well that she omitted thyroid, a reversion in symptoms to the former myxedematous state occurred. Her serum proteins at this time were 8.4 per cent. After 15 days of thyroid (0.5 grains daily) her proteins did not change but there was a weight loss of 1 kgm. Thyroid was increased to 1 grain daily and 14 days later the serum proteins were the same, yet loss of weight amounted to 1.6 kgm, the basal metabolic rate had risen, cholesterol had fallen, and most of the symptoms of myxedema had gone. It was not until 3 months after treatment was begun that the proteins fell to below 8.0 per cent. In the first 2 patients omission of thyroid produced a rise in serum proteins and resumption of thyroid

a diagnostic aid in doubtful or atypical cases. Of the 16 patients in Tables I and II who improved markedly on $\frac{1}{2}$ to $2\frac{1}{2}$ grain doses of thyroid, only 1 had a cholesterol as low as 316 mgm per cent. In this latter case the results were moderate because complicating disorders overshadowed the hypothyroid symptoms. However the most useful criterion suggested by this study lies in the fact that not one of the patients with many symptoms attributable to hypothyroidism but with serum cholesterol within normal limits was improved by thyroid. For practical purposes a serum cholesterol below 275 mgm per cent indicates that in most instances the patient will not be benefitted by thyroid. This criterion must be used with caution, particularly in the case of borderline figures ranging between 275 and 300 mgm per cent. The present study for example shows that no one with a cholesterol below 316 mgm per cent improved with thyroid therapy. It is conceivable however, that a patient who in the beginning of the development of hypothyroidism might have had a cholesterol over 350 mgm per cent would later, as a result of severe malnutrition (32) or liver disease have this reduced to 275 mgm per cent in spite of the continued progress of the hypothyroid disease. That malnutrition may materially interfere with the development of a high cholesterol in myxedema is suggested by the studies on patient A52821 Figure 9. When first seen she was malnourished and had a microcytic anemia as well as fully-developed myxedema, and her cholesterol was 318 mgm per cent. Subsequently she made a good recovery on 1 grain of thyroid, but after 3 years of good health stopped taking thyroid. When she returned again with about the same degree of myxedema as during the first study she was not malnourished and it is noteworthy that her serum cholesterol was 550 mgm per cent in contrast to the previous 318 mgm per cent. Patients A62475 and A58944 had moderate degrees of microcytic hypochromic anemia, a condition often associated with a reduction in cholesterol. It is therefore not surprising that the severity of the myxedema cannot be shown to be quantitatively proportional to the concentration of cholesterol.

The relation of the serum proteins to the clinical state of the 5 myxedematous patients in group 1 remains obscure. It is evident that the proteins

were elevated to high normal or slightly above when the patients were myxedematous, and that they gradually fell as improvement followed the administration of thyroid. But they did not necessarily fall with the disappearance of edema. In the case of A45359, Figure 5 proteins had declined only slightly when the edema had practically disappeared. In patients A52821, Figure 9 and A58944 Figure 8 the proteins actually rose as weight loss occurred the edema disappeared and there was striking clinical improvement. In the remaining 2 only did the proteins and weight fall simultaneously. These divergent responses could be explained by Gibson's studies. He found in 2 cases of myxedema that, after thyroid administration and after increases in basal metabolic rate the blood volume increased in one patient and remained unchanged in the other (29). In these 2 patients the level of serum proteins would probably not have shown similar changes after thyroid administration.

In 10 instances, in which both albumin and globulin were determined simultaneously with a fall of serum proteins following thyroid both fractions were about equally implicated 7 times, globulin was more important twice and albumin more important once. Since the 2 patients whose globulin fell more markedly than the albumin had several complications, and since the patient whose albumin fell more than the globulin showed only a small decrease in protein and albumin near the error of the method, only little significance can be attributed to these exceptions. The simultaneous fall in total proteins albumin and globulin of the 7 patients would imply an increase in blood volume and hydration.

Further investigation may show that although serum proteins are not as sensitive an indicator of deficiency in thyroid hormone as the basal metabolic rate and cholesterol the finding of values of over 7.7 per cent may constitute an additional and useful criterion of hypothyroidism. However it is clear from the studies of the patients in group 1 and 2 that many patients with definite myxedema may have low normal proteins and that a fall in proteins may not occur until some time after clinical improvement, rise in basal metabolic rate, and fall in cholesterol have taken place. One could not expect in clinical cases of this kind an exact correlation between proteins

Turner and Steiner (20) reported a sharp drop in serum cholesterol in 10 patients given 30 to 240 milligrams of thyroid per day for 6 weeks. Of their 10 subjects 2 had diabetes, 6 had various forms of heart or vascular disease, and 5 had cholesterols in the control period above 265 mgm per cent so that the individuals can hardly be judged as uncomplicated. It is also impossible to tell whether the cholesterols fell for a short time or persistently throughout the thyroid administration. Cohen and Fierman (23) gave 8 male schizophrenic patients 6 to 15 or 18 grains of Armour's USP desiccated thyroid for approximately 95 days, and after 67 days repeated the dosage for 69 days. Although no figures for cholesterol are included in the paper, these authors found "no marked displacement of the seasonal trends" of cholesterolemia. Schmidt and Hughes (24) found that thyroxin given orally did not alter the plasma, whole blood total or free cholesterols of normal dogs but did reduce the plasma total and free cholesterol of thyroidectomized dogs. Houchin and Turner (25) noted that, 22 to 30 hours after 5 milligrams of thyroxin had been injected into rabbits, the blood fats decreased. Hurxthal and Perkins (26) have shown that desiccated thyroid diminishes the total cholesterol in the whole bodies of mice. The responses of different animals to thyroid or thyroxin are somewhat difficult to relate to the effect of thyroid on the serum cholesterols of humans because there seem to be certain animals, rabbits or rats fed on normal diets, which do not develop hypercholesterolemia after thyroidectomy (27, 28). Also, the effect of thyroxin on serum cholesterol has not been investigated in the present study. It is apparent from the evidence cited here that thyroid, in sufficiently large doses, does lower the serum cholesterol of humans, other than nephrotics, but that in subjects without thyroid deficiency the actual decreases may be small, and that some regulatory reaction may soon permit the cholesterol to return to its normal level. On the other hand, in patients with either spontaneous or post-thyroidectomy myxedema, very small doses of thyroid, $\frac{1}{2}$ to 2 grains, rapidly reduce the hypercholesterolemia.

These data demonstrate that the levels of serum cholesterol and other lipid fractions are markedly increased by a deficiency in thyroid hormone. The

concentrations of lipoids may be 2 to 3 times as great as the normal. This increase in serum lipoids greatly exceeds any effects of hemoconcentration due to the state of myxedema. The blood volume in 7 myxedematous patients has been shown by Gibson to be about 15.5 per cent below normal (29). When thyroid is administered to a patient with hypothyroidism, a fall in lipoids is one of the early changes that takes place. These points are well illustrated in the individual charts of patients in group 1 and in Figures 2, 5, 6, 7, 8, and 9. The sensitiveness of serum cholesterol to deficiency in thyroid hormone is exemplified by patient A45359, Figure 5. After thyroidectomy this patient was apparently doing well, showed no signs of myxedema, and the basal metabolic rate was plus 4, yet the cholesterol rose to 471 mgm per cent. Only 2 weeks later the symptoms of myxedema began to appear, and in 4 weeks the syndrome was full blown. By this time the cholesterol was 603 mgm per cent, the basal metabolic rate minus 27 per cent, and the proteins 8 per cent. Thus slow development of clinical symptoms has been pointed out by Means and Lerman (30). On treatment the fall in cholesterol paralleled the remission in symptoms. The marked reversion in symptoms, lipoids and basal metabolic rate to the pre-treatment levels in 3 myxedematous patients, A45359, A43137, A52821, on omission of thyroid, conclusively demonstrates their dependence on the amount of thyroid hormone. These results in general agree with those of Hurxthal and coworkers (3, 31, 5).

In a patient who has a basal metabolic rate below minus 20 per cent the finding of a cholesterol of over 300 mgm increases the probability of thyroid deficiency. But certain complications such as nephrosis, common bile duct obstruction, or xanthomatosis, which may elevate the serum lipoids, must be ruled out before thyroid administration is advocated. Furthermore, examination of P1667, 85071 and group 4 reveals that there may be other not clearly-defined exceptions. These patients showed no improvement in symptoms on thyroid therapy, although there was a rise in basal metabolic rate and a fall in cholesterol and proteins.

The results indicate therefore that high serum cholesterol is a sufficiently consistent phenomenon in hypothyroidism to warrant its determination as

- 5 Mason, R. L., Hunt, H. M. and Hurxthal L. M., Blood cholesterol values in hyperthyroidism and hypothyroidism—their significance. *New England J Med.*, 1930 203, 1273
- 6 Gardner J. A., and Gainsborough H. The relation ship of plasma cholesterol and basal metabolism. *Brit. M. J.*, 1928, 2, 935
- 7 Boyd, E. M., and Connell W. F., Thyroid disease and blood lipids. *Quart. J Med.*, 1936 NS 5 455
- 8 Boyd, E. M., and Connell, W. F., Plasma lipids in diagnosis of mild hypothyroidism. *Quart. J Med* 1937 6 467
- 9 Grabfield, G. P., and Campbell, A. G., Note on relation between blood cholesterol and basal metabolic rate. *New England J Med.*, 1931 205 1148
- 10 McGee, L. C., Blood cholesterol in disturbances of basal metabolic rate. *Annals Int. Med.* 1935 9, 728.
- 11 Peters J. P., and Eisenman, A. J. Serum proteins in diseases not primarily affecting cardiovascular system or kidneys. *Am J M Sc.*, 1933 186, 808
- 12 Bruckman F. S., D'Esopo L. M., and Peters, J. P., Plasma proteins in relation to blood hydration. IV Malnutrition and serum proteins. *J Clin. Invest.*, 1930 8, 577
- 13 Bogdanovitch, S. B. and Man E. B., Effects of castration theelin testosterone and antuitrin S on lipoids of blood, liver and muscle of guinea pigs. *Am. J Physiol.*, 1938, 122 73
- 14 Man E. B., and Peters J. P., Gravimetric determination of serum cholesterol adapted to Man and Gildea fatty acid method, with note on estimation of lipid phosphorus. *J Biol. Chem.*, 1933 101, 685
- 15 Man E. B., Note on stability and quantitative determination of phosphatides. *J Biol. Chem.*, 1937, 117, 183
- 16 Man, E. B., and Gildea, E. F. Modification of Stoddard and Drury titrimetric method for determination of fatty acids in blood serum. *J Biol. Chem.* 1932, 99 43
- 17 Man E. B. and Gildea E. F. Notes on extraction and saponification of lipids from blood and blood serum. *J Biol. Chem.* 1937 122, 77
- 18 Man, E. B., and Gildea, E. F. Variations in lipemia of normal subjects. *J Biol. Chem.* 1937 119 769
- 19 Sperry W. M. Concentration of total cholesterol in blood serum. *J Biol. Chem.*, 1937 117 391
- 20 Turner K. B., and Stelner A., Long term study of variation of serum cholesterol in man. *J Clin. Invest.*, 1939 18, 45
- 21 Page, I. H., and Farr, L. E., Influence of high and low fat diets and thyroid substance on plasma lipids of nephrotic patients. *J Clin. Invest.*, 1936 15, 181
- 22 Blumgart H. L., and Davis D., Hypothyroidism induced by complete removal of normal thyroid gland in treatment of chronic heart disease. *Endocrinology* 1934 18, 693
- 23 Cohen, L. H., and Fierman J. H., Metabolic, cardiovascular and biochemical changes associated with experimentally induced hyperthyroidism in schizophrenia. *Endocrinology* 1938, 22, 548
- 24 Schmidt, L. H., and Hughes, H. B., Free and total cholesterol content of whole blood and plasma as related to experimental variations in thyroid activity. *Endocrinology* 1938 22, 474
- 25 Houchin, O. B., and Turner C. W., The relation of the pituitary to blood lipids. *Endocrinology*, 1939 24, 638.
- 26 Hurxthal, L. M., and Perkins, H. J., The destruction of total body cholesterol by the feeding of desiccated thyroid. *Lahey Clinic Bull.*, 1938 1 19
- 27 Leonard, S. L. The blood cholesterol in thyrotoxicomized rats as related to the effectiveness of gonadotropic hormones. *Endocrinology*, 1939 24, 679
- 28 Turner K. B., Present, C. H., and Bidwell, E. H., Role of thyroid in regulation of blood cholesterol of rabbits. *J Exper. Med.*, 1938 67, 111
- 29 Gibson, J. G., 2d, and Harris A. W., Clinical studies of blood volume. V. Hyperthyroidism and myxedema. *J Clin. Invest.*, 1939 18, 59
- 30 Means, J. H., and Lerman, J. The symptomatology of myxedema, its relation to metabolic levels, time intervals and rations of thyroid. *Trans. Assoc. Am. Physicians*, 1934, 49 214
- 31 Hurxthal L. M., and Hunt, H. M. Clinical relationships of blood cholesterol with summary of our present knowledge of cholesterol metabolism. *Annals Int. Med.*, 1935 9, 717
- 32 Man E. B., and Gildea, E. F., Serum lipoids in malnutrition. *J Clin. Invest.*, 1936, 15 203
- 33 Gildea, E. F., Kahn, E., and Man, E. B., Relationship between body build and serum lipoids and discussion of these qualities as pyknicophilic and leptophilic factors in structure of personality. *Am. J Psychiat.*, 1936, 92 1247

and evidences of thyroid activity, because proteins are subject to the influence of so many nutritional factors. The exact relation between proteins and thyroid function could only be elucidated by controlled experiments.

Patients with diminished basal metabolic rates, low serum lipoids, and incapacitating fatigability are still being studied. These patients fall into a different classification than the patients discussed by Hurxthal (4), who has considered the effects of the pituitary and adrenals in lowering the basal metabolic rate. From the study of the patients in groups 3 and 4, it is apparent that the administration of thyroid does not ameliorate the symptoms of lack of energy and multiple complaints. On the other hand, patients with low basals and low lipoids often are asthenic in physique (33) and are troubled by tension, irritability, tremulousness and insomnia. The administration of thyroid to such persons seems contraindicated, as for example in (6) Table III. This man had found work difficult because of weakness and easy fatigability. When it was discovered that his basal was minus 27 per cent he began to take thyroid. The results were disappointing and the dose was rapidly increased to 4 grains and was maintained for 1 month. As improvement was negligible he tried 6 grains daily. This resulted in tremor and nervousness with no relief of symptoms although the basal metabolic rate had risen to minus 1 per cent. The dose was reduced to 4 grains and maintained for 3 months. He continued to feel as weak as ever. Thyroid was stopped. After a month his basal had fallen to minus 22 per cent, and his weakness and fatigability had not increased. It is of particular interest that the cholesterol was extremely low (149 mgm per cent) without thyroid, and that with thyroid it was reduced to the remarkable level of 94 mgm per cent which has been found previously only in grave malnutrition, cirrhosis and acute and severe hyperthyroidism.

CONCLUSIONS

1 The levels of serum cholesterol, phosphatides, and fatty acids are readily affected by changes in the amount of thyroid hormone in the body.

2 Patients with myxedema have remarkably high serum lipoids (cholesterol 335 to 603 mgm

per cent). Amelioration in symptoms by administration of 1 to 2 grains of desiccated thyroid is closely paralleled by a fall in lipoids and a rise in basal metabolic rate. The lipoids revert to former high levels with return of symptoms following omission of thyroid therapy. In patients without hypothyroidism thyroid lowers serum cholesterol, but the decreases are not so significant and larger amounts are required.

3 The level of serum cholesterol is a useful tool in determining the presence or absence of hypothyroidism. A serum cholesterol below 275 mgm per cent practically excludes hypothyroidism and indicates that the administration of thyroid will probably have little or no effect in relieving symptoms.

4 Although the combination of high cholesterol and low basal metabolic rate may be the result of conditions other than hypothyroidism, patients with these findings should be considered as having hypothyroidism until the contrary is proven by their failure to improve after treatment with thyroid.

5 Serum proteins tend to lie above or in the upper part of the normal range in hypothyroidism. The level of proteins could not be consistently correlated with the amount of edema. In many of these patients nutritional and metabolic factors appeared to be more important than changes in water balance in determining proteins.

It would have been impossible to collect this material if it had not been for the clinical services of Dr. Paul Lavietes and Dr. Alexander Winkler under whose care were many of the patients.

BIBLIOGRAPHY

- 1 Bronstein, I. P., Studies in cretinism and hypothyroidism in childhood. I. Blood cholesterol. *J. A. M. A.*, 1933, 100, 1661.
- 2 Gilligan, D. R., Volk, M. C., Davis, D., and Blumgart, H. L., Therapeutic effect of total ablation of normal thyroid on congestive heart failure and angina pectoris. VIII. Relationship between serum cholesterol values, basal metabolic rate and clinical aspects of hypothyroidism. *Arch. Int. Med.*, 1934, 54, 746.
- 3 Hurxthal, L. M., Blood cholesterol and thyroid disease. III. Myxedema and hypercholesteremia. *Arch. Int. Med.*, 1934, 53, 762.
- 4 Hurxthal, L. M., Blood cholesterol and hypometabolism, suprarenal and pituitary deficiency, obesity and miscellaneous conditions. *Arch. Int. Med.*, 1934, 53, 825.

THE EFFECT OF PROLONGED ADMINISTRATION OF SULFANILAMIDE ON RATS WITH NEPHROTOXIC NEPHRITIS

By JOSEPH E. SMADEL AND HOMER F. SWIFT

(From the Hospital of The Rockefeller Institute for Medical Research, New York City)

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Treatment with sulfanilamide is sometimes indicated in patients suffering from nephritis or in those with infections which may lead to renal disease, hence, it is desirable to know whether a damaged kidney has an increased susceptibility to this drug. Several investigators (1, 2) have reported that administration of sulfanilamide to normal rats over long periods in daily amounts somewhat greater than those used for man, is not associated with the development of renal lesions but there is not complete agreement on this point (3). For a general discussion of the toxic effects of sulfanilamide the recent review of Marshall (4) may be consulted.

The present work was undertaken to determine whether rats with experimental glomerulonephritis will tolerate sulfanilamide¹ when given in doses comparable to those usually employed for human beings. Since it is possible to alter significantly the course of nephrotic nephritis in rats by feeding different diets (5) it was proposed to employ this procedure to influence the renal changes toward regression or progression in rats with induced nephritis. Thus, animals with different degrees of renal disease would be available for testing the effect of sulfanilamide in the injured kidney.

An unexpected factor, *i.e.*, heredity entered into the experiment and necessitated a redivision of the original groups of rats for an adequate interpretation of results; consequently, the number of animals in each of the final groups was small. Nevertheless, the uniform response of the rats to the drug justifies a report of the observations.

EXPERIMENTAL METHODS

Anti kidney serum.—This serum was prepared in rabbits by the intraperitoneal injection of suspensions of

perfused kidney tissue obtained from rats of the Whelan strain. Anti serum number 4557 used in preceding experiments (5) was injected intravenously into rats in a dosage of 0.75 cc. per 100 grams of body weight. This total amount was given in three divided doses on consecutive days. Most of the animals received an additional 0.25 cc. of anti-kidney serum five weeks after the original injection.

Strains of rats.—Only a few hooded rats of the so-called Whelan strain which we had used throughout our earlier work were available when the present investigation was undertaken. As a result, two members of this inbred line, of opposite sexes, were placed in each of six groups and in addition twenty hooded rats of the Evans strain were used to complete the requisite number. Animals weighing 50 to 60 grams when received were observed for a week and subjected to repeated urine analyses. The animals received the initial injection of nephrotoxin when they weighed about 75 grams.

Care of animals.—Rats were kept in separate jars and fed one of two purified diets. The constituents in these rations have been described elsewhere (5), and it is sufficient to say here that only the protein and carbohydrate contents were varied. The high protein diet contained 40 per cent lactalbumen and 29 per cent of a mixture of karo powder and cane sugar; the low protein diet contained 5 per cent protein and 64 per cent carbohydrate.

Urine analyses and determination of body weight were made on alternate days for the first six weeks and once or twice weekly thereafter.

Rats were sacrificed three months after nephritis had been induced and complete autopsies were performed. Sections for microscopic study were always prepared from kidney and heart and usually from the pancreas and brain. Paraffin sections of kidney tissue were stained by the usual methods and, in addition, by Mallory's aniline blue stain and McGregor's modification (6) of the Mallory Heidenhain technique.

Administration of sulfanilamide and estimation of its excretion.—Sulfanilamide was usually given by mouth, but when quantitative estimations of urinary excretion were made the drug was injected subcutaneously. Stock solutions for use by either route were prepared by dissolving the drug in boiling Ringer's Solution and, after cooling, adding sufficient sterile diluent to bring the concentration of sulfanilamide to 5 mgm. per cc. For oral administration a proper amount of the stock solution of drug *i.e.* 10 cc. per 100 grams of body weight, was placed in the individual drinking bottle of each rat and 30 cc. of a 10 per cent solution of karo syrup in water

¹ The sulfanilamide used was the brand known as Protynin, kindly supplied by the Winthrop Chemical Company Inc.

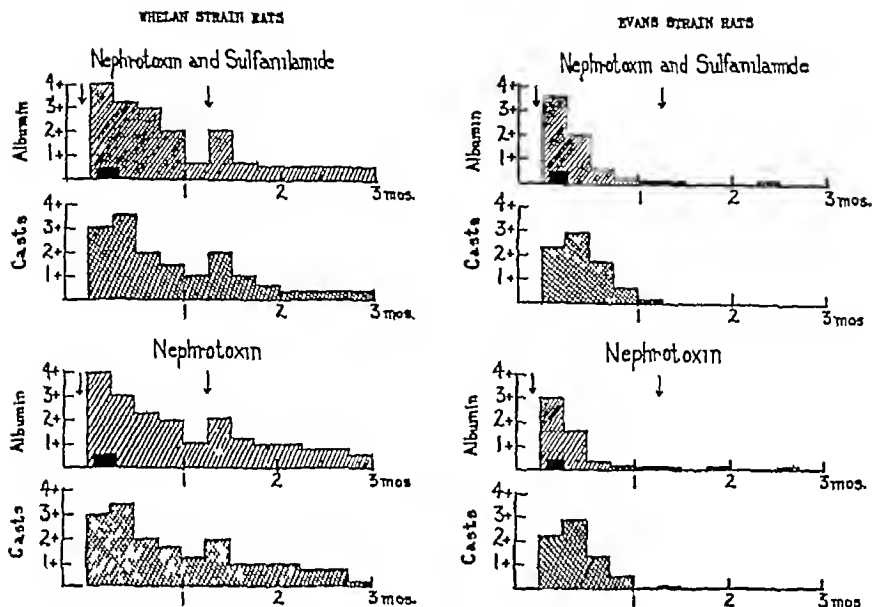


FIG. 1 EFFECT OF SULFANILAMIDE ON COURSE OF NEPHROTOXIC NEPHRITIS IN RATS MAINTAINED ON A LOW PROTEIN DIET

Arrows indicate injection of nephrotoxin. Initial amount of anti kidney serum was 0.75 cc. per 100 grams of body weight the second dose was 0.25 cc. per 100 grams of body weight. The presence of edema is represented by the solid black areas.

ferences were noted on pathological examination. The graphs in Figures 1 and 2 summarize the urinary findings in the nephritic animals which received the drug in each diet and strain group, as well as in the controls receiving no drug.

A second set of control rats which did not receive nephrotoxin excreted normal urine throughout the period while they received sulfanilamide. Moreover, no changes were observed in their kidneys on microscopic examination except those which have been recorded in normal animals maintained on these two diets (5).

There was no significant difference in the excretion of sulfanilamide by normal and nephritic rats fed the low protein diet. The average values for the entire period were 70 per cent in the former group and 73 per cent in the latter. The results obtained in the high protein diet group were more variable and may indicate a diminution

in excretion of the drug by animals with induced renal injury during the second week after injection. Nevertheless, the average excretion values in these groups of normal and nephritic animals were not greatly different, being 81 per cent and 70 per cent, respectively. The amount of free drug in the urine, determined before hydrolysis, was in general about 35 per cent of the total sulfanilamide excreted. The results of observations on the urinary excretion of sulfanilamide are summarized in Figure 3.

Retention of sulfanilamide, which may occur in patients with severe nephritis (9), was not noted in our animals, however, none of the rats developed renal failure during the relatively short course of the experiment. Urine collected before each excretion test, i.e. approximately sixty hours after the last administration of the drug contained very small amounts of sulfanilamide, only

were added. Animals took this mixture avidly and rarely wasted more than a few drops.

The method of determining the urinary excretion of sulfanilamide was similar to that employed by Marshall and Cutting (7) and was carried out as follows. On Monday morning a sample of urine was collected and examined in order to be certain that the last dose of the drug, given on Friday, was no longer being excreted in appreciable amounts. Late that afternoon each rat was injected subcutaneously with a proper amount of the stock solution of sulfanilamide, placed in a metabolism jar for collection of urine, and given a drinking bottle containing 15 cc. of diluted Karo to insure an adequate volume of urine. The next morning, sixteen and one-half hours after injection, the residual urine was pressed from the rat's bladder and the total volume was measured. An aliquot portion of the sample of urine was used to determine the concentration of sulfanilamide. The urinary excretion of the drug was expressed as the percentage of the injected amount which was excreted in both the free and combined form over a period of sixteen and one-half hours. Estimations of free and combined sulfanilamide were made on urine and blood by the techniques of Marshall (8).

Grouping of experimental animals—Severe glomerulonephritis was induced in twenty-four young rats by means of anti-kidney serum. Half of the animals were then placed on a low protein diet while the remainder were given a high protein diet. Six of the nephritic animals in each diet group received sulfanilamide in doses of 0.05 gram per kilo of body weight on five consecutive days each week for a period of ten weeks, the other rats with nephritis were kept as controls. Eight additional control animals which did not receive nephrotoxin were treated with sulfanilamide, these also were distributed in equal groups and given one of the two diets. Each of the six groups of animals consisted of two rats of the Whelan strain and two or four of the Evans strain.

RESULTS

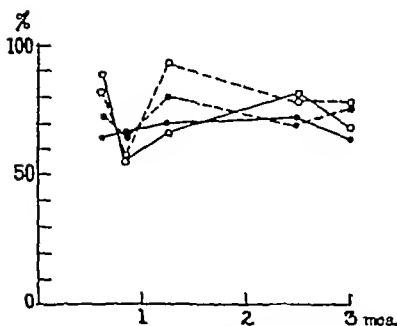
Differences in the response of Whelan and Evans rats to nephrotoxin A striking difference in the course of the nephritis in the two strains of rats was apparent by the end of the first month. Animals of the Whelan strain behaved as in previous experiments (5), *i.e.*, the nephritis rapidly subsided and became of mild intensity in the rats fed the low protein diet, while in the high protein diet group the nephritis continued to be severe. On the other hand, the acute nephritis induced in the Evans strain of rats by nephrotoxin rapidly subsided irrespective of the diet fed. These findings are clearly shown in Figures 1 and 2, in which graphic representations are presented of the average urinary albumen and casts excreted by the members of the different groups.

Since the nephritis subsided in most of the animals more rapidly than we had originally anticipated, all rats, except those of the Whelan strain maintained on a high protein diet, were given a second series of injections of nephrotoxin, this second dosage consisted of one-third of the amount of the first dose and was given five weeks after the initial injection. In order to avoid fatal anaphylactic reactions, the animals were first desensitized by injecting 0.05 cc. of anti-kidney serum, on the following day the remainder of the nephrotoxin was given. This procedure was apparently successful with the Whelan strain of rats since neither injection was followed by any anaphylactic reaction. The Evans strain of rats, however, almost invariably developed signs of a slight or moderate reaction after each injection. These observations, which apparently indicate that the former strain was less sensitive to rabbit serum than the latter, suggest an explanation for the different renal response of the two rat strains to reinjection. For example, circulating antibody in rats sensitive to rabbit serum might combine with the rabbit nephrotoxin and thus prevent its acting on the kidney. The second course of nephrotoxin was followed by a moderate exacerbation of nephritis in the animals of the Whelan strain and a slight recurrence in members of the Evans strain of rats fed the high protein diet, however, no appreciable increase of renal irritation was observed in reinjected rats of the Evans strain which were being fed the low protein diet.

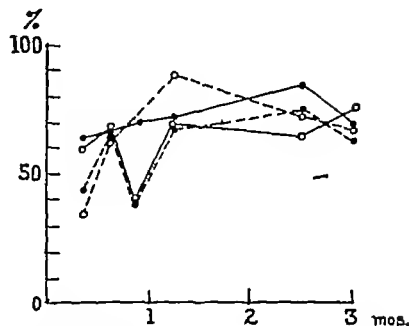
Additional observations on the various responses of different inbred strains of rats to nephrotoxin will be reported in the near future. It may be mentioned here, however, that the histological evidence for recovery from renal injury observed in rats of the Evans strain fed the high protein diet is similar in many respects to that previously noted in animals of the Whelan line maintained on a low protein diet (5).

Administration of sulfanilamide to normal and nephritic rats The administration of sulfanilamide in the manner described above to nephritic rats had no obvious effect on the course of the renal disease. In every instance the degree of urinary abnormality and the rate of growth were practically identical for rats of the same strain in a given diet group, whether sulfanilamide was ingested or not. Furthermore, no apparent dif-

LOW PROTEIN DIET GROUP



HIGH PROTEIN DIET GROUP



- Normal Whelan Rats receiving Sulfanilamide
 ○—○ Nephritic Whelan Rats receiving Sulfanilamide
 ●—● Normal Evans Rats receiving Sulfanilamide
 ●—● Nephritic Evans Rats receiving Sulfanilamide

FIG. 3. URINARY EXCRETION OF SULFANILAMIDE BY NORMAL AND NEPHRITIC RATS MAINTAINED ON DIFFERENT DIETS

Nephritis was induced March 26 1937 Sulfanilamide, begun ten days later was given on five consecutive days each week in doses of 0.05 gram per kilo of body weight. Per cent excretion of sulfanilamide represents the proportion of drug free and combined, in the urine secreted during sixteen and one half hours after administration.

drug to normal rats for considerable periods. The inference is clear, therefore, that neither normal nor diseased renal tissue of rats is detectably injured by the necessity for excreting sulfanilamide in moderate amounts. This is in contrast to the recently recorded observations made on rats treated with sulfapyridine (10 11) which inflicted definite renal damage.

The ability of the nephrotoxin injured rats' kidneys to excrete sulfanilamide at a normal rate may be contrasted with the lowered capacity of human nephritic kidneys to excrete this drug (9). That this indicates an essential qualitative difference between human nephritis and that induced in the rats is doubtful, for renal failure was not present in these rats while it has been observed in patients who failed to excrete the drug normally.

CONCLUSIONS

Sulfanilamide, given in therapeutic doses for long periods to rats with nephrotoxic nephritis,

did not affect the course of the experimental disease.

Rats with nephrotoxic nephritis, but without renal failure, excreted sulfanilamide in the same amounts as did normal rats. Incidentally, it was found that rats of the Whelan and Evans strains respond differently to the effect of nephrotoxic serum and diet.

BIBLIOGRAPHY

- 1 Marshall E. K., Jr. Cutting, W. C., and Emerson, K., Jr., Toxicity of sulfanilamide. *J. A. M. A.*, 1938, 110 252.
- 2 Molitor H. and Robinson, H., Some pharmacological and toxicological properties of sulfanilamide and benzylsulfanilamide. *J. Pharmacol. and Exp. Therap.* 1939 65 405.
- 3 Davis, H. A., Harris, L. C., Jr., and Schmeisser H. C. Tissue changes following prolonged administration of sulfanilamide in rats. *Arch. Path.* 1938, 25 750.
- 4 Marshall E. K., Jr., Bacterial chemotherapy Pharmacology of sulfanilamide. *Phys. Rev* 1939 19 240.

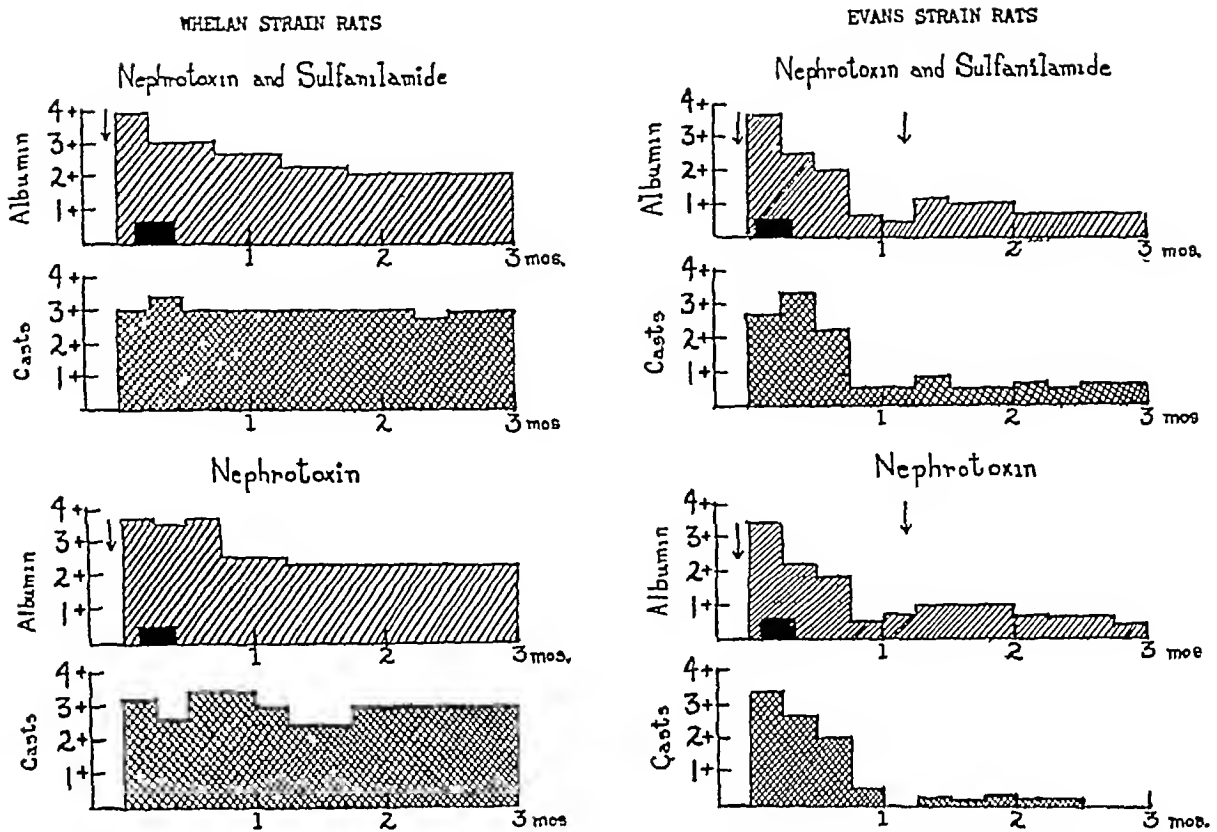


FIG 2 EFFECT OF SULFANILAMIDE ON COURSE OF NEPHROTOXIC NEPHRITIS IN RATS MAINTAINED ON A HIGH PROTEIN DIET

Arrows indicate injection of nephrotoxin. Initial amount of anti-kidney serum was 0.75 cc. per 100 grams of body weight. Evans rats received a second dose of 0.25 cc. per 100 grams of body weight. The presence of edema is represented by the solid black areas

on a few occasions was a concentration of 0.01 mgm per cent recorded. Furthermore, at the end of the experiment, each rat was injected subcutaneously with an amount of drug equal to 15 mgm per 100 grams of body weight and bled from the heart sixteen hours later. No notable variation in the amount of drug was detected in the blood of nephritic and control animals of a given diet group, but a considerable difference in the blood level was observed in rats fed the high and low protein diets. Thus, the values 3.4 and 2.6 mgm per cent represented the average blood level of sulfanilamide in the nephritic and control rats fed the high protein diet, while 10.9 and 10.5 mgm per cent were the average values for the two groups of animals fed the low protein diet. An explanation of this difference is not at hand. However, the excretion of larger volumes of urine by rats on the high protein diet may have

been a factor in reducing the blood level of the drug in this group. The results on the whole are similar to those obtained by Marshall and Cutting in their studies on excretion of sulfanilamide by normal rats (7).

DISCUSSION

The present experiments indicate that rat kidneys, which have been subjected to various degrees of damage by a combination of nephrotoxic serum and injurious or beneficial diets, are not appreciably affected by sulfanilamide given over relatively long periods in daily dosage of approximately 0.05 gram per kilo of body weight, the usual maximal dosage for man. These observations, moreover, are in accord with those of the majority of workers who could find no significant changes in the kidneys after administering this

CALCIUM EXCHANGE OF THE INTESTINE IN EXPERIMENTAL HYPERTHYROIDISM¹

By T. L. ALTHAUSEN, WM. J. KERR AND M. STOCKHOLM

(From the Department of Medicine, University of California Medical School, San Francisco)

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The work of Parhon (1) and of Aub, Bauer, Heath and Ropes (2) established the existence of a negative calcium balance in experimental and in clinical hyperthyroidism. Pugsley and Anderson (3, 4) and Cope and Donaldson (5) among others confirmed this finding in rats and in human beings, respectively, and in addition, in agreement with Aub (6) stressed the prominent part which loss of calcium in the feces plays in hyperthyroidism, in contrast to other diseases characterized by a negative calcium balance. Hansman and Wilson (7) and Hansman and Fraser (8) recently showed that occasionally calcium equilibrium may be present in clinical hyperthyroidism and that the intestine need not always be the principal route of excretion of calcium.

Kummer (9) suggested that diminished absorption of dietary calcium accounted for the high calcium content of feces in patients with hyperthyroidism. On the other hand Aub *et al.* (2) demonstrated the occurrence of osteoporosis in patients with hyperthyroidism and obtained data on the simultaneous increase in excretion of phosphorus which "was quantitatively such as to suggest that most of the calcium excreted came from tertiary calcium phosphate in the bones." This and certain negative data influenced Albright, Bauer and Aub (10) to postulate that the thyroid hormone exerts a specific catabolic action on the metabolism of calcium phosphate in bones. Recently the possibility that in hyperthyroidism there may be an "inability to assimilate the calcium of the food quite apart from the mobilization of calcium from the bones" was again brought up by Hansman and Fraser (8).

Most investigators in this field were chiefly interested in the endogenous metabolism of calcium and conducted their studies with diets very low

in calcium in order to eliminate the factor of unabsorbed dietary calcium. However, this experimental arrangement does not distinguish between increased excretion of calcium into the intestine and decreased re-absorption of excreted calcium. Also apparently no attempts have been made to study quantitatively whether diminished absorption of calcium from the food could be a factor contributing to the loss of calcium from the body in hyperthyroidism. The latter possibility presented itself prominently when we discovered that intestinal absorption of carbohydrates and of fatty acids was greatly increased in hyperthyroidism (11) suggesting that absorption of calcium may be decreased in this disease through preferential absorption of other food elements.

For these reasons, we undertook to study in testinal absorption and excretion of calcium in normal and in hyperthyroid rats. An additional incentive for this study was furnished by a suggestion of Albright *et al.* (10) based on the work of Petersen and Levinson (12) that increased tissue permeability might be the cause of increased calcium excretion. If increased permeability of the intestinal mucosa were the cause of increased diffusion of calcium from the blood into the intestine one would expect to find also an abnormally high rate of diffusion of calcium in the reverse direction provided that its concentration in the intestine exceeded that in the blood.

Just this was reported by Guassardo and Peola (13) who studied the absorption of calcium chloride from an isotonic solution by means of a double Vella fistula in 2 dogs before and after administration of thyroxine. In three control experiments each lasting 30 minutes, their dogs absorbed on the average 39 and 37 per cent of the calcium introduced into the intestinal loop. In two similar experiments on each dog performed 20 minutes after intravenous injection of 1.1 mgm of thyroxine (the dogs weighed about 4.5 kgm), they absorbed on the average 59 and 60 per cent, respectively, of the introduced calcium.

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² Read before the Annual Meeting of the American Association for the Study of Goiter at Cincinnati, May 22 to 24, 1939.

- 5 Farr, L. E., and Smadel, J. E., Influence of diet on course of nephrotoxic nephritis in Rats. *Proc. Soc. Exp. Biol. and Med.*, 1937, 36, 472
- Smadel, J. E., and Farr, L. E., Effect of diet on pathological changes in rats with nephrotoxic nephritis. *Am. J. Path.*, 1939, 15, 199
- 6 McGregor, L., Finer histology of normal glomerulus. *Am. J. Path.*, 1929, 5, 545
- 7 Marshall, E. K., Jr., and Cutting, W. C., Absorption and excretion of sulfanilamide in mouse and rat. *Bull. Johns Hopkins Hosp.*, 1938, 63, 328
- 8 Marshall, E. K., Jr., Determination of sulfanilamide in blood and urine. *J. Biol. Chem.*, 1937-38, 122, 263
- 9 Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., Para-aminobenzene-sulfonamide absorption and excretion. Method of determination in urine and blood. *J. A. M. A.*, 1937, 108, 953
- 10 Gross, P., Cooper, F. B., and Lewis, M., Urinary concretions caused by sulfapyridine. *Proc. Soc. Exp. Biol. and Med.*, 1939, 40, 448
- 11 Antopol, W., and Robinson, H., Urolithiasis and renal pathology after oral administration of 2(sulfanilylamino) pyridine (sulfapyridine). *Ibid.*, 1939, 40, 428

tate. From a comparison of these figures it can be concluded that hyperthyroidism does not materially affect the intestinal absorption of calcium in fasting rats when an excess of calcium lactate is present.

b Absorption on a calcium free diet In this experiment normal and hyperthyroid rats were fed on a calcium free diet adequate in other respects and containing 26 per cent of fat for 2 days before the absorption experiment. They were not fasted and had access to food throughout the absorption experiment. Figures for absorption of calcium in both groups of rats fed on the calcium free diet were very similar, and are given in Table II. From a comparison of these figures with those obtained from fasting rats that received the same dose of calcium lactate, it is seen that the presence of food does not significantly decrease the absorption of calcium in either normal or hyperthyroid rats when an excess of calcium lactate is present in the intestine.

Since rats feed chiefly during the night while our absorption experiments were started in the morning and carried out during the day it was thought that the small decrease in absorption of calcium seen in fed as compared to fasting hyperthyroid rats might be rendered significant by simultaneously administering some food. Accordingly the calcium lactate was given to 6 hyperthyroid rats in a 20 per cent solution of dextrose without altering the absorption of calcium.

c The effect of phlorizin on absorption It has been suggested by Verzar and McDougal (16) that the formation of calcium phosphorus compounds in the intestinal mucosa may play a part in the absorption of calcium. In view of our previous finding that phosphorylation in the in-

testine is altered in hyperthyroidism it seemed of interest to learn whether inhibition of intestinal phosphorylation would affect the absorption of calcium. For this purpose normal rats were given two different amounts of calcium lactate with 50 mgm. of phlorizin in the solution. The figures in Table III show that administration of phlorizin did not affect the absorption of calcium.

2 Intestinal excretion of calcium

a Excretion during fasting Young normal and hyperthyroid rats were fasted in a metabolic cage for 30 hours before determinations of calcium in the washings from the entire gastro-intestinal tract were made. The figures for the amount of calcium present in the digestive tract of these rats are given in Table IV. From these figures it is clear that the intestine of hyperthyroid rats contained no more excreted calcium than that of normal rats.

b Excretion on a calcium free diet In this experiment the rats were fed a calcium free diet as mentioned previously. Determinations of calcium in the contents of the entire digestive tract were made and are recorded in Table IV. From these figures it is seen that the presence of food increased the amounts of excreted calcium in the intestines of both normal and hyperthyroid rats. This was somewhat more marked in the hyperthyroid group.

With the object of possibly intensifying this finding by more prolonged administration of thyroxin, determinations of excreted calcium into the intestine were performed in 8 rats injected with thyroxin for 3 weeks and in 4 rats similarly treated for 1 month instead of for 12 days. The severity of hyperthyroidism in the last group of

TABLE III
Effect of phlorizin on absorption of calcium

Normal rats					Rats treated with phlorizin				
Number of rats	Range of original weight	Loss of weight after fasting 24 hours	Dose of calcium per 100 grams of weight	Amount of calcium absorbed per 100 grams of weight	Number of rats	Range of original weight	Loss of weight after fasting 24 hours	Dose of calcium per 100 grams of weight	Amount of calcium absorbed per 100 grams of weight
11	grams 168-208	per cent 6.5	mgm. 21.35	mgm. 9.61 ± 0.96*	3	grams 200-208	per cent 8.5	mgm. 21.95	mgm. 10.61 ± 0.02*
10	grams 150-180	per cent 7.6	mgm. 26.44	mgm. 15.02 ± 2.08	3	grams 180-208	per cent 2.9	mgm. 28.28	mgm. 14.57 ± 2.82

* Standard deviation.

TABLE I
Absorption of calcium in normal rats during fasting

Number of rats	Range of original weight	Loss of weight after fasting 24 hours	Dose of calcium per 100 grams of weight	Amount of calcium absorbed in 6 hours		Number of rats	Range of original weight	Loss of weight after fasting 24 hours	Dose of calcium per 100 grams of weight	Amount of calcium absorbed in 18 hours	
				Per 100 grams of weight	Per cent					Per 100 grams of weight	Per cent
6	grams 160-200	per cent 5.2	mgm 14.79	mgm $6.01 \pm 1.23^*$	40		grams	per cent	mgm	mgm	
11	168-208	6.5	21.35	9.61 ± 0.96	45	4	138-160	6.2	18.37	$13.91 \pm 1.34^*$	77
10	150-180	7.6	26.44	15.02 ± 2.08	58	3	155-176	7.7	23.60	17.19 ± 0.99	72
						3	165-174	7.1	35.78	30.26 ± 3.27	84

* Standard deviation

EXPERIMENTS

1 Intestinal absorption of calcium

a Absorption during fasting After being fasted in a metabolic cage for 24 hours in order to clear the intestine of food residues, young rats were given by stomach-tube several measured amounts of a 10 per cent solution of calcium lactate. After 6 hours in some cases, and 18 hours in others, the animals were sacrificed, and the contents of the entire digestive tract were used for determinations of residual calcium. Figures for absorption of calcium by normal rats are given in Table I. In this table it is seen that the absorption of calcium under our experimental conditions was approximately proportional to the dose of calcium lactate, and that its rate increased somewhat with the size of the dose. On the other hand, extension of the time allowed for absorp-

tion of a given amount of calcium lactate from 6 to 18 hours resulted in comparatively little additional absorption, so that about 20 per cent of the administered calcium still remained in the digestive tract at the end of 18 hours. A similar observation was made by Cohn and Greenberg (14) in regard to the absorption of phosphorus.

A number of rats were rendered hyperthyroid by intraperitoneal injections of 0.1 mgm of thyroxin per 100 grams of body weight given daily for 12 days. The average basal metabolic rate of rats so treated, as determined in a closed circuit apparatus similar to that used by Benedict and Macleod (15), is increased about 50 per cent. These rats were then used for the absorption experiment as described.

Table II gives figures for the absorption of calcium by the hyperthyroid rats and by the corresponding controls for each dose of calcium lac-

TABLE II
Absorption of calcium in normal and in hyperthyroid rats during fasting and on a calcium-free diet

Dietary status	Ab-sorption time	Normal rats					Hyperthyroid rats				
		Num-ber of rats	Range of original weight	Loss of weight after fasting 24 hours	Dose of calcium	Calcium ab-sorbed per 100 grams of weight	Num-ber of rats	Range of original weight	Loss of weight after fasting 24 hours	Dose of calcium	Calcium ab-sorbed per 100 grams of weight
Fasting	hours 6	11	grams 168-208	per cent 6.5	mgm 21.35	mgm $9.61 \pm 0.96^*$	10	grams 178-228	per cent 14.6	mgm 21.00	mgm $11.13 \pm 0.39^*$
	6	10	150-180	7.6	26.44	15.02 ± 2.08	12	160-192	14.9	27.71	16.29 ± 1.49
	18	3	165-174	7.1	36.78	30.26 ± 3.27	4	208-260	13.9	33.22	29.61 ± 1.15
Calcium free diet	6	8	160-210	0.0	21.14	8.58 ± 1.07	10	200-232	8.9	22.46	8.89 ± 1.29

* Standard deviation

TABLE IV

Intestinal excretion of calcium in normal and in hyperthyroid rats during fasting and on a calcium free diet

Dietary status	Normal rats				Hyperthyroid rats			
	Number of rats	Range of original weight	Loss of weight after fasting 30 hours	Amount of calcium found in the intestine	Number of rats	Range of original weight	Loss of weight after fasting 30 hours	Amount of calcium found in the intestine
Fasting	10	grams 160-212	per cent 7.9	mgm $2.36 \pm 0.5^*$	10	grams 165-200	per cent 15.5	mgm $1.95 \pm 0.46^*$
Calcium free diet	10	170-230	0.0	3.94 ± 0.28	20	200-313	10.2	4.69 ± 0.74

* Standard deviation

animals is shown by the fact that 5 out of 9 rats died from hyperthyroidism before the end of the month. However, the amounts of calcium found in their intestines were practically the same as those in rats treated with thyroxin for the usual 12 days. For this reason, in Table IV the figures for all fed hyperthyroid rats were combined.

3 Fecal excretion of calcium

a The influence of thyroxin The calcium content of feces was determined over 2-day periods in rats receiving our calcium-free diet for 3 and for 10 days, respectively, previous to collection, before and after 3 weeks of injections with thyroxin. Figures for the average daily fecal output

of calcium under these conditions are given in Table V. These figures show that the fecal output of calcium of rats deprived of calcium for 3 days was more than twice that of rats that were on a calcium-free diet for 10 days, and that administration of thyroxin increased fecal excretion of calcium 98 and 100 per cent, respectively, in the two groups.

b The rôle of increased food intake Since our hyperthyroid rats ate about twice the normal amount of food (8 grams per 100 grams of weight as against a normal of 4 grams), it was felt that the greater volume of food passing through their digestive tracts might be a factor in decreasing re-absorption of calcium excreted into the intes-

TABLE V

Influence of thyroxin, of food intake, and of intestinal peristalsis on fecal excretion of calcium

Thyroid status	Experimental condition	Rats deprived of calcium for 3 days before stool collection			Rats deprived of calcium for 10 days before stool collection		
		Number of rats	Range of weight	Daily fecal calcium	Number of rats	Range of weight	Daily fecal calcium
Normal	Food intake 4 grams*	10	grams 185-231	mgm $4.47 \pm 0.52^\dagger$	4	grams 220-275	mgm 2.04
Hyperthyroid	Food intake 8 grams*	10	187-227	8.85 ± 1.26	4	220-275	4.09
Hyperthyroid	Food intake 4 grams*	10	190-222	5.79 ± 0.35			
Normal	Administration of castor oil	10	193-230	7.77 ± 0.99	4	180-211	4.01
Normal	Administration of cascara	10	187-245	7.86 ± 0.74			
Hyperthyroid	Administration of morphine	10	185-240	5.51 ± 0.38			

* Per 100 grams of weight

† Standard deviation

important part in the increased fecal excretion of calcium by hyperthyroid rats was demonstrated by our experiment in which slowing of intestinal peristalsis with morphine markedly reduced the calcium content of the feces in such animals

The presence of increased intestinal peristalsis in patients with hyperthyroidism is well known both through radiologic observations and the occurrence of diarrhea in severe cases. The same phenomenon was also observed experimentally (19). The opposite, namely, abnormally sluggish intestinal peristalsis is characteristic of myxedema in which there is also a markedly diminished fecal output of calcium. In our rats injected with thyroxin, a tendency to diarrhea was also noted. It was brought out particularly when somewhat irritating substances such as xylose, phlorizin or oleic acid were administered (11). In the experiment where a mixture of oleic acid and phlorizin had to be given, all hyperthyroid rats developed diarrhea and we were forced to control this tendency with morphine before administering the mixture. Under similar circumstances, no normal rats developed diarrhea.

Whether overeating or hyperperistalsis has a greater influence on fecal excretion of calcium is a difficult problem to solve. On the one hand we obtained more decisive experimental results by producing increased peristalsis. On the other hand a greater volume of food would not only cause the diffusion of more calcium from the blood but would also stimulate peristalsis.

If we accept hyperperistalsis and overeating as the chief causes of increased fecal output of calcium in hyperthyroidism, we are able to account for several clinical and experimental observations heretofore unexplained. (1) Our theory accounts for the loss of calcium through the bowel in hyperthyroidism in the absence of hypercalcemia. (2) It explains why hyperthyroidism is the only disease characterized by a negative calcium balance in which loss of calcium in the feces plays a prominent part. (3) It explains the occasional absence of increased fecal calcium in hyperthyroidism (7, 8) by the fact that this is not a primary feature of the disease but depends on the presence of increased intestinal peristalsis and overeating in themselves secondary manifestations of hyperthyroidism that may occasionally be

absent. (4) In conjunction with the mechanism of intestinal absorption discussed above, our theory accounts for the absence of fecal loss of calcium in hyperparathyroidism and other diseases characterized by hypercalcemia and a negative calcium balance but lacking increased intestinal peristalsis and overeating. In these conditions, an abnormally high level of calcium in the blood presumably leads to increased diffusion of calcium into the small intestine, but later this excess of calcium is re-absorbed in the colon with little or no resultant loss of calcium in the feces. (5) It probably accounts for the finding of Pugsley (20) that a negative calcium balance due to increased fecal output of calcium can be produced experimentally in rats by oral administration of very large doses of dinitrophenol. While preparing rats for an absorption experiment with administration of dinitrophenol (11) by stomach tube twice a day in doses much smaller than the ones used by Pugsley, it was observed that many of our animals developed spontaneous diarrhea and could not be used for the intended purpose. The probability that hyperperistalsis rather than stimulation of metabolism was responsible for the increased fecal excretion of calcium obtained by Pugsley is enhanced by his inability to produce a rise in fecal calcium with subcutaneous or intraperitoneal injections of dinitrophenol which would obviate local irritation of the digestive tract. It is also significant that Robbins (21) found the excretion of calcium and phosphorus to be normal in patients receiving clinical doses of dinitrophenol that raised the basal metabolic rate to 37 per cent plus but presumably were not large enough to cause intestinal irritation. Our theory that intestinal hyperperistalsis plays an important part in the fecal loss of calcium in hyperthyroidism is supported by a recent article of Meulengracht (22) in which he describes the occurrence of osteomalacia of the spine due to daily use of cathartics over a long period of time.

In view of our discovery that increased intestinal peristalsis and overeating play an important part in the increase of fecal excretion of calcium in hyperthyroidism, it will be interesting to see whether increased elimination of calcium in the urine, which takes place in this condition in spite of a normal or practically normal calcium level

tion of calcium are determined not alone by laws of simple diffusion, if phosphorylation plays any role in its passage through the intestinal mucosa, was ruled out by the experiment in which phlorizin was used to inhibit intestinal phosphorylation without any change in the amount of absorbed calcium. Incidentally, this experiment also proves conclusively that phlorizin has no depressing effect on intestinal absorption in general.

The fact that the absorption of calcium lactate was not diminished in fed hyperthyroid rats indicates that no impairment in the intestinal absorption of dietary calcium exists in experimental hyperthyroidism due to increased absorption of competing foodstuffs or any other cause, provided that an abundance of calcium is present in the intestine.

Turning to the question of intestinal excretion of calcium, we must consider the mechanism of intestinal absorption. According to Verzar and McDougal (16), the present conception of intestinal absorption by simple diffusion is that, during passage through the small intestine, the ions present in its contents and those found in the blood plasma reach an equilibrium due to a two-way exchange of electrolytes between the lumen of the intestine and the capillaries. When the fluid contents of the small intestine enter the colon, they are subjected to hydrostatic pressure which forces part of the water into the capillaries, rendering the remaining mixture hypertonic. This causes the diffusion of more electrolytes into the blood. The indicated process goes on until the food residues assume a solid consistency and are expelled as feces with little loss of valuable salts from the body. This conception in application to the calcium exchange of the intestine is supported by the finding that, contrary to the opinion of earlier authors, no calcium is excreted into the colon (17, 18).

In the light of this conception, we would expect increased diffusion of calcium from the blood into the small intestine only when the calcium level of the blood is increased. This is found in hyperthyroidism only to a slight degree or not at all (2, 6), and therefore can hardly be a major factor in the loss of calcium through the bowel, especially since in hyperparathyroidism, where the blood calcium is definitely elevated, there is no excessive

excretion of calcium in the feces. On the other hand, the fact that there is no active excretion of calcium into the intestine in hyperthyroidism, over that expected through normal diffusion from the blood, is demonstrated by the equal amounts of calcium found in the intestine of fasting normal and hyperthyroid rats. It is in these animals that one would expect to obtain the greatest difference in this respect if active excretion were taking place, because fasting has an inhibiting influence on peristalsis which is more active in hyperthyroidism, and therefore in fed rats would tend to minimize the difference by sweeping out some of the extra calcium.

There remain two explanations for the increased fecal output of calcium in hyperthyroidism. The first of these is that the approximately double amount of food passing through the digestive tract of hyperthyroid rats may interfere with normal re-absorption of excreted calcium. This possibility was suggested by our finding that both normal and hyperthyroid rats maintained on a calcium-free diet had more calcium in their intestinal contents than the fasting animals, and that this finding was more marked in hyperthyroid rats. The experiment in which the food of hyperthyroid rats was limited to the amount usually consumed by normal animals shows that on a calcium-free diet an increased intake of food plays a definite part in the loss of calcium through the bowel, although it does not account for all of it.

The second explanation is that increased intestinal peristalsis accelerates the passage of feces through the colon to such an extent as to interfere with normal re-absorption of excreted calcium. This possibility was suggested by the fact that the fecal excretion of calcium by hyperthyroid rats is markedly increased without a corresponding increase in the calcium content of the intestine. Our experiments with administration of sub-purgative doses of castor oil or of cascara to normal rats at two levels of fecal excretion of calcium³ show that the endogenous calcium in the feces is markedly raised by intestinal hyperperistalsis, and that this factor alone could account for the total increase of fecal calcium in hyperthyroidism. That peristalsis actually does play an

³ Following deprivation of calcium for 3 and for 10 days, respectively. (See Table V)

- tions of large intestine of man in absorption and excretion. *Arch. Int. Med.*, 1936 58 1095
- 18 Johnson, R. M., Absorption and excretion of calcium and phosphorus in three patients with colostomy and ileostomy. *J. Clin. Invest.*, 1937 16 223
- 19 Morrison, S., and Feldman, M., An experimental study of the effect of the thyroid on the motility of the gastro-intestinal tract. *Am. J. Digest. Dis.*, 1939 6 549
- 20 Pugsley L. I., Effect of 2,4-dinitrophenol upon calcium, creatine and creatinine excretion in rat. *Biochem. J.*, 1935 29 2247
- 21 Robbins, C. L., Effect of dinitrophenol on calcium and phosphorus metabolism. *J. Nutrition* 1935, 10 187
- 22 Meulengracht E., Osteomalacia of spine following abuse of laxatives. *Lancet*, 1938, 2 774

of the blood, can also be explained on the basis of an acceleration of the urinary flow, an increase in the intake of water, or both. Experiments to test these possibilities are under way.

Our data on the calcium exchange of the intestine do not contradict the extensive investigations of calcium metabolism in hyperthyroidism performed by Aub and his co-workers, which led to the conclusion that the source of the calcium that is being lost in this condition is the tertiary calcium phosphate from the bones. But they furnish an explanation of the mechanism by which the loss of calcium in the feces takes place, creating the necessity of drawing on the calcium reserves of the skeleton. Our experiments are also of help in reconciling the often contradictory data obtained when some workers study the calcium metabolism in hyperthyroidism under conditions of a low calcium intake while others allow an adequate dietary calcium.

SUMMARY

1 Administration of thyroxin or of subpurgative doses of castor oil or of cascara to rats approximately doubled the fecal excretion of calcium.

2 Restriction of the food intake or administration of morphine markedly decreased the fecal output of calcium in hyperthyroid rats.

3 Increased permeability of the intestinal mucosa, active excretion of calcium, and increased absorption of food elements enjoying preferential intestinal absorption were ruled out as factors causing loss of calcium through the feces in hyperthyroidism.

4 From these findings it is concluded that hyperperistalsis and overeating are the chief causes of increased fecal output of calcium in hyperthyroid rats maintained on a calcium-free diet.

5 The proposed theory of increased fecal excretion of calcium in hyperthyroidism accounts for several heretofore unexplained clinical and experimental observations in conditions associated with a negative calcium balance.

6 There is no appreciable change in calcium absorption in hyperthyroidism.

BIBLIOGRAPHY

- 1 Parhon, M., L'influence de la thyroïde sur le métabolisme du calcium. *Compt. Rend. Soc. de Biol.*, 1912, 72, 620.
- 2 Aub, J. C., Bauer, W., Heath, C., and Ropes, M., Studies of calcium and phosphorus metabolism. III Effects of thyroid hormone and thyroid disease. *J. Clin. Invest.*, 1929, 7, 97.
- 3 Pugsley, L. I., and Anderson, E. M., Effect of desiccated thyroid, irradiated ergosterol and ammonium chloride on excretion of calcium in rats. *Biochem. J.*, 1934, 28, 754.
- 4 Pugsley, L. I., and Anderson, E. M., Effect of administration of calciferol on increased calcium excretion induced by thyroxine. *Biochem. J.*, 1934, 28, 1313.
- 5 Cope, O., and Donaldson, G. A., Relation of thyroid and parathyroid glands to calcium and phosphorus metabolism. *J. Clin. Invest.*, 1937, 16, 329.
- 6 Aub, J. C., Calcium and phosphorus metabolism. *Harvey Lectures*, series XXIV, 1928-1929, p. 151.
- 7 Hansman, F. S., and Wilson, F. H., Calcium and phosphorus metabolism in diseases of the thyro-parathyroid apparatus. I Calcium, phosphorus and total metabolism in hyperthyroidism and part played by parathyroid glands. *M. J. Australia*, 1934, 1, 37.
- 8 Hansman, F. S., and Fraser, W. A., Calcium and phosphorus metabolism in diseases of thyro-parathyroid apparatus. II Calcium and phosphorus balance (a) following therapeutic radiation of hyperplastic thyroid gland, and (b) in hyperthyroidic patients treated with iodine. *J. Clin. Invest.*, 1938, 17, 543.
- 9 Kummer, R. H., Recherches sur le métabolisme minéral dans la maladie de Basedow. *Rev. méd. de la Suisse Rom.*, 1917, 37, 439.
- 10 Albright, F., Bauer, W., and Aub, J. C., Studies of calcium and phosphorus metabolism. VIII Influence of thyroid gland and parathyroid hormone upon total acid-base metabolism. *J. Clin. Invest.*, 1931, 10, 187.
- 11 Althausen, T. L., and Stockholm, M., Influence of thyroid gland on absorption in digestive tract. *Am. J. Physiol.*, 1938, 123, 577.
- 12 Petersen, W. F., and Levinson, S. A., Skin reactions, blood chemistry, and physical status of "normal" men and of clinical patients. *Arch. Path.*, 1930, 9, 151.
- 13 Guassardo, G., and Peola, F., Influenza di alcuni ormoni sull'assorbimento intestinale del Ca e K. *Pathologia*, 1930, 22, 455.
- 14 Cohn, W. E., and Greenberg, D. M., Studies in mineral metabolism with aid of artificial radioactive isotopes. I Absorption, distribution, and excretion of phosphorus. *J. Biol. Chem.*, 1938, 123, 185.
- 15 Benedict, F. A., and Macleod, F., Heat production of albino rat. I Technique, activity control, and influence of fasting. *J. Nutrition*, 1929, 1, 343.
- 16 Verzá, F., and McDougall, E. J., Absorption from the Intestine. Longmans, Green and Co., London, New York and Toronto, 1936.
- 17 Welch, C. S., Wakefield, E. G., and Adams, M., Func-

DIET AND DEATH IN ACUTE UREMIA¹

BY T. ADDIS AND W. LEW

(From the Department of Medicine, Stanford University Medical School, San Francisco)

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In 1930 (1) a new way to produce a reversible acute uremia was described. The vena cava is tied above the entrance to the renal veins. In the rat this induces such an extreme congestion of the kidneys that the secretion of urine stops. The venous blood from the kidneys is obliged to run down the vena cava to the pelvis and find its way back to the heart through veins in the abdominal wall. The enlargement of these veins can be followed from day to day since they are visible through the peritoneum when the abdomen is opened. As this compensatory venous return develops, the congestion of the kidney decreases until on the second day after operation the secretion of a small amount of dilute urine begins. It contains a high concentration of protein and many renal failure casts. But the rate of excretion of urea is still deficient so that it is not until the third day that the blood urea concentration, now at very high levels—frequently more than 400 mgm per 100 cc.—begins to decrease. Thereafter the rates of protein, cast, and cell excretion diminish until by the seventh day after operation there is no longer any anatomical or functional evidence of a renal lesion. In all cases the rats with tied vena cavae look sick, take very little food or water and frequently have a tremor of the entire body, exaggerated on effort. Some of them die at the height of the toxic state.

There are advantages in working with a form of uremia relatively simple in its pathogenesis and reversible by natural means but experience has shown that valid conclusions with respect to the effect of any experimental factor can be drawn only if there is rigid uniformity with respect to the operative technique, the sex, age and general condition of the rats that are used. The operation itself is done in less than a minute. The abdomen is opened by cutting with scissors along the right costal margin. The right kidney is pulled downwards with iris forceps and, with a curved

needle a thread is passed round the vena cava and tied. One stitch is needed for the muscles and the skin is closed with clips. In the experiments reported here only females were used. The mortality increases with age. In this work the age of the rats was 125 days. The animals used were of the Slonaker strain from which the Wistar Institute colony was derived. They had been reared under quite constant conditions on the same diet. They were not at all disturbed by handling. This may be an important variable, for all of a small series of rather wild frightened rats operated on in another laboratory died almost at once with signs of venous congestion of the lower limbs.

Death in uremia constantly follows cessation or any continued extreme decrease in the secretion of urine, and so the cause is presumably the retention of some urinary constituent. But, since injections of such substances as urea or creatinine are ineffective it is often supposed that the causative agent may be some as yet unidentified urinary constituent, probably some nitrogen-containing derivative of protein metabolism. If this hypothesis is correct, change in the rate of protein metabolism induced by variation of the amount of protein consumed as food by altering the concentration of the hypothetical toxic substance, should induce corresponding differences in the number of animals dying from uremia after the operation.

In constructing the diets used in these experiments it was thought that derivatives of protein metabolism associated with the proteins in certain foods might be important. Thus casein is a relatively pure protein whereas, when meat is taken there is with the protein a not inconsiderable quantity of nucleic acid and its derivatives along with creatin and many known and unknown nitrogen-containing substances that are not proteins. So we ground up fresh meat liver and kidney and dried them quickly in a current of warm air. These air-dry foods were made up in 70 per cent

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A STUDY OF ACUTE RENAL INSUFFICIENCY¹

By HYMAN C. BERGMAN AND D. R. DRURY

(From the Department of Physiology School of Medicine University of Southern California, Los Angeles)

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In the work reported in this paper we were concerned with factors which affect the survival time of totally nephrectomized rats. The purpose of this is to obtain knowledge about the causes of death in acute anuria and to get information which would be of value in the treatment of acute anuria. Such information can be applied in the treatment of chronic renal insufficiency and will also to some extent aid in the understanding of the mechanisms of the disturbances in this condition. One need only to refer to the recent review of Harrison and Mason (1) to see what a complicated condition chronic uremia may be. Acute anuria is undoubtedly much simpler and one is justified in considering each separately.

The method of investigation was simple. We nephrectomized groups of rats and determined the effects of different experimental conditions and agents on the survival time of the animals. We have attempted to vary only one factor at a time by having a group of control animals treated exactly as the experimental group except for the one variable that was being investigated. The variables studied were age, sex, food and the administration of certain substances which were suggested by the results of the feeding experiments.

Control groups and effect of sex and age

Our first concern was to determine the best experimental conditions, the variability in controls, and the effect of ordinary physiological variants such as age, sex and nutritional state. For age and sex 4 groups were taken. Group 1 96-day old females, Group 2 99-day-old males, Group 3, females over 200 days old, Group 4 males over 200 days old. All rats were fasted 24 hours before operation but were allowed water of which they drank very little. No food or water was

given after nephrectomy. Prior to this they had been on a stock diet. This contained yellow corn meal, whole wheat flour, dried milk and the necessary salts and vitamins. The average weights of the groups were as follows: Group 1 (20 rats), 84 grams; Group 2, (21 rats), 89 grams; Group 3 (20 rats) 161 grams; Group 4, (16 rats), 239 grams. The survival times are plotted in Figure 1. Groups 1 and 2 survived an average of 40 and 44 hours compared to 75 and 82 hours for Groups 3 and 4. It is quite apparent that young rats cannot survive total nephrectomy as well as adult rats. Sex has little effect on survival time, females living slightly shorter periods than males.

Preoperative fasting period

The effect of preoperative fast was determined in 3 groups of adult males. Group 4 (reported above) was fasted 24 hours. Group 5 was fasted 48 hours and Group 6 received the stock diet up to the time of operation. Neither food nor water was given after operation. Group 5 (18 rats) had an average weight of 260 grams and Group 6 (20 rats) 252 grams. The survival times are plotted in Figure 1. The average survival times for 0, 24-, and 48-hours fast are 79, 82, and 94 hours respectively. Group 7 illustrates the effect of water administration after nephrectomy. Five cc. distilled water was given by stomach tube to these rats every 24 hours. There is a definite shift of the entire group towards shorter survival as compared to Group 4 and the averages of the 2 groups are 67 hours and 81 hours. Considering the closeness of the grouping in the 2 groups one is justified in concluding that water in this amount (about half the normal intake of rats) is harmful here. This group serves as our control for all experimental groups which were given solutions postoperative. The volume of the solutions given in these cases was about the same as that of the water in this group.

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tomized rats during one hour before operation. If these latter animals had eaten as much meat as the former, undoubtedly their life would have been much shorter due to the combined action of the large amount of potassium and of the other toxins.

SUMMARY

The low variability of the survival time of the standard nephrectomized rat makes it an excellent test object for the study of the toxic mechanisms in renal insufficiency and for the study of the efficacy of different therapeutic measures in this condition.

The conditions for the test animals must be very closely controlled as certain physiological factors affect this survival time. Immature rats have definitely shorter survival times than the

adult. Fasting brings about a slight prolongation of life. Water (in an amount approximating the ordinary intake of the rat) is deleterious when given after nephrectomy. Glucose is beneficial.

Meat (fed immediately before operation) is definitely toxic.

The urine of fasted rats contains toxic materials but the amount of these poisons is not enough to account quantitatively for the entire toxic condition which supervenes after nephrectomy.

Potassium is harmful and is responsible for part of the toxicity of meat.

BIBLIOGRAPHY

1. Harrison, T. R., and Mason, M. F., Pathogenesis of uremic syndrome. *Medicine*, 1937 16 1.
2. Fishberg A. M., Hypertension and Nephritis. Lea and Febiger Philadelphia, 1935 3rd Edition.

the urine of meat-fed rats is potassium. The toxicity of potassium seems to be roughly proportional to the amount given. Potassium is not markedly toxic when given in smaller amounts. When only one dose of the above solution (17 mgm potassium per 100 grams of rat) was given to 5 rats, the average survival time was 57 hours, and when 6 mgm per 100 grams of rat were given the average survival time (8 animals) was 69 hours.

It is apparent that potassium is toxic to nephrectomized animals and undoubtedly a definite part of the toxicity of meat in this condition is due to this element. But the entire toxicity cannot be ascribed to it. Examination of the results will show this. As stated previously, under effect of feeding, the group of animals fed meat immediately before nephrectomy, ate an average of 5 grams and the average survival time was 39 hours. The average potassium intake per rat with this meat would be 15 mgm and the average per 100 grams of rat would be 10.2 mgm. The effect of intake of such an amount of potassium would be expected to give a survival time between that of the 2 groups mentioned above (6 mgm—69 hours and 17 mgm—57 hours) or something like 63 hours as compared to the actual survival of 39 hours. On the other hand, examination of our results with regard to the toxic factor in the permutit-treated urine of meat-fed animals reveals that potassium is the main source of the toxic activity there. These two statements are not necessarily irreconcilable. All the toxic agents of meat may not be present in the permutit-treated urine of meat-fed rats. The kidneys may dispose of some of the toxic products of meat in other ways than just by excretion of them. Then again ammonia may play an important rôle in this toxicity, and finally it is possible that the permutit removed other toxic materials along with the ammonia.

DISCUSSION

Our purpose in this paper is not to solve with any simple formula the riddle of uremia. A reading of the reviews of Harrison and Mason (1) and of Fishberg (2) will convince one that the problem is very complicated and will probably need years of careful systematic research to unravel completely. Primarily we wish to present

here a method and to show its fitness for the investigation of certain phases of this problem and its use in obtaining information of value in the therapy of renal insufficiency. The low variability in the survival time of the standard nephrectomized rat under any well-defined set of conditions makes it easy to determine whether any given factor or procedure is harmful or beneficial in renal insufficiency. One has the advantage of simplicity in dealing with acute insufficiency rather than chronic insufficiency since the chronic state is definitely more complicated than that following nephrectomy. This method should be of value too in studying those mechanisms present in both acute and chronic uremia.

Our results with the urine-fed animals indicate that the detoxifying function of the kidney is carried out to a considerable extent by excretion of the toxic products in the urine. That certain of the ordinary products of normal metabolism are toxic and are eliminated as such in the urine follows from our observation that the urine of fasted animals shortens the life of the experimental animals. It is not quantitatively so, however, since, when we gave the urine of normal fasting rats excreted during 48 hours to the nephrectomized rats, it did not shorten their lives 48 hours but 17 hours (these animals received about the same amount of water with the urine as did the control group, *i.e.*, their average survival times were 50 hours as compared to the control group's 67 hours). We cannot, then, think of the toxic products of normal metabolism entirely as simple poisons that are produced at a certain rate and excreted in the urine as such, nor can we think that if they are dammed back when the kidney is not excreting them they will kill the animal when their concentrations reach a critical value. The mechanism is not as simple as that and we intend continuing our investigation of this phase of the problem.

Our results indicate that potassium is toxic to nephrectomized rats, that meat feeding is also harmful, and that part of this action of meat is attributable to the potassium it contains. The urine of meat-fed rats is highly toxic and the chief poison in it (after permutit treatment) is potassium. Our meat-fed rats from which we were collecting urine ate per day an average of twice as much meat as did the meat-fed nephrec-

CHANGES IN THE GLUCOSE TOLERANCE OF OBESE SUBJECTS AFTER WEIGHT REDUCTION

BY ROGER S. HUBBARD AND EDGAR C. BECK

(From the Buffalo General Hospital and the University of Buffalo Medical School Buffalo)

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In a previous publication (1) a summary of studies made upon obese subjects was presented. Included in this article was a discussion of the changes in blood sugar concentration which followed the ingestion of 100 grams of glucose by patients who were overweight. These patients showed a somewhat low tolerance for sugar, an observation which is in accord with that of a number of investigators (2, 3, 4, 5). After dietary therapy had been instituted and continued for some time the patients showed some improvement in the tolerance tests, but the change was slight and was not considered definitely significant. During the past four years the number of patients studied has increased greatly and a fair proportion has been reduced approximately to normal weight by dietary therapy. It has seemed desirable to examine the question again at this time for in several recent papers (6, 7, 8) it has been suggested that reduction of weight has a definite effect upon the glucose tolerance.

The literature upon the glucose tolerance test is extensive. It has been reviewed recently by Herrmann (9) and by Myers and McKean (10) and will not be discussed here in detail. There are, however, a few points more or less closely connected with the subject of this communication which should be emphasized. Not only does a large number of pathological states affect the test, but certain physiological conditions may also influence it (11). Among the non pathological causes of variations, age, the presence of various emotional states, including fatigue and the diet taken prior to the test may be mentioned specifically. It is not strange therefore, that Lennox (12) and others have observed rather marked irregularities in the results of repeated glucose tolerance tests upon the same subject. The occurrence of these makes it necessary it seems to the authors to collect data suitable for statistical analysis before valid conclusions can be drawn concerning the effect of any procedure upon glucose tolerance curves.

The existence of some relationship between obesity and diabetes seems to be quite generally accepted (13). This relationship has led many investigators to study the glucose tolerance of obese subjects. The results of these studies have been somewhat confusing. A few cases of hypoglycemia have been noted in such patients (14, 15, 16) but high blood sugar values have been observed much oftener (5). Frequently there is some other condition present, such as advanced age or hypertension, which appears to have a direct influence upon glucose metabolism. This often makes a study of the specific effect of obesity difficult (5, 17) and some authors have not found satisfactory evidence that obesity itself is closely associated with abnormalities of glucose tolerance (18). Since the results upon untreated obese subjects appear to vary markedly, it is evident that the interpretation of changes after any therapy is difficult.

There are few papers in which the effect of diet and weight reduction is so reported as to be comparable with the results obtained in this clinic. In 1931 Tyner (19) stated that the rise in blood sugar following Brills test meal (20) was less marked after 12 patients had been on a restricted diet than it was when they were first seen. In three more recent papers (6, 7, 8) other experiments are described. In each of these later studies attention was centered upon the dietary treatment of patients selected because some abnormality of the glucose tolerance test was found in a preliminary examination. Each author shows clearly that dietary therapy producing loss of weight was accompanied in these subjects by an improvement in the glucose tolerance. In one of these papers (8) by Newburgh and Conn it is suggested that this improvement is directly associated with the change in weight. Since only patients with abnormal initial tests were included in these studies it is difficult to determine whether the findings are applicable to the average obese subject.

were not markedly so. The results of the separate tests upon the 39 patients in the series were compared with normal values for the procedure used as given in a recent paper by McCullagh and Johnston (24). In 27, or 69 per cent of the subjects, the fasting blood sugar was higher than 120 mgm per 100 cc., the value which these authors accept as normal. Eighty seven per cent of the patients showed some abnormality but in most instances, the degree of the abnormality was not marked. In 5 of the tests all four blood sugar values were within normal limits. In 9 the fasting blood sugar alone was high and in only 7 were all four blood sugar concentrations above normal.

It seemed desirable to determine whether a relationship between the degree of the obesity and the relatively slight abnormality shown by the glucose tolerance tests could be demonstrated. Accordingly the correlation coefficient between the sum of the four blood sugar values and the per cent by which the patients exceeded their ideal weight was calculated. This coefficient was $+0.08 \pm 0.11$. It has been suggested by Ogilvie (5) and Newburgh and Conn (8) that the duration of the obesity indirectly affects the tolerance for glucose and that the age of the patients serves as a rough index of the time during which the patients have been overweight. The correlation coefficient between the age and the sum of the four blood sugar values was therefore calculated. It was $+0.17 \pm 0.10$. Of the 5 patients with completely normal glucose tolerance tests 3 were below and 2 above the average of the ages for the whole series. Of the 7 patients 30 years old or less only 2 showed glucose tolerance curves which were completely normal. Although no positive evidence of a correlation between either the degree or duration of the obesity and the changes in the glucose tolerance curves of these patients was demonstrated nevertheless the authors believe that there probably is some relationship between the obesity and the changes in the tolerance test. There seems to be no other possible explanation for the occurrence of such a large number of abnormal values in patients selected as these patients were.

In Table II are given the results of glucose tolerance tests before and after weight reduction by the dietary therapy described. There is no

TABLE II

Comparison of glucose tolerance tests carried out before and after weight reduction

Blood specimens analyzed	Extreme range		Median values		Standard deviations	
	Before weight reduction	After weight reduction	Before weight reduction	After weight reduction	Before weight reduction	After weight reduction
Specimens were taken before glucose was given	104-147	100-145	127.0 \pm 9.0	114 \pm 1.4	14 \pm 1.1	10 \pm 0.8
1 hour after glucose	146-266	112-212	182 \pm 8.3	161 \pm 4.3	23 \pm 2.7	22 \pm 1.4
1 hour after glucose	128-294	101-278	184 \pm 8.3	148 \pm 3.1	19 \pm 1.0	23 \pm 1.9
2 hours after glucose	106-254	84-195	154 \pm 4.6	122 \pm 3.8	24 \pm 1.6	25 \pm 1.3

* Under before weight reduction are the averages of the results of glucose tolerance tests made when the 39 patients studied were first seen.

† Under after weight reduction are the values obtained by an identical procedure after the weights of the patients had been reduced to approximately normal values.

Average values were also computed. They were not significantly different from the median values given and have therefore been omitted.

doubt that the values found after treatment were much lower than those obtained when the patients were first seen, i.e. that an improvement in tolerance had taken place. The total improvement can be conveniently expressed by calculating the decrease in the sum of the four blood sugar values. The average and median values of this decrease were 115 ± 9 and 130 ± 11 mgm. per 100 cc.

Changes of this order of magnitude are certainly too marked to be attributed to the ordinary causes which produce fluctuations in the glucose tolerance test. They must be associated with some factor which is common to the whole series of patients. Only two such factors seem worth considering. These are the changes in the weight of the patients and the low diet which was fed during the period of therapy. It has been reported that each of these may affect the glucose tolerance test. An attempt was made to determine whether there was any evidence of an effect of either of these two factors upon the glucose tolerance test. The relationship between the change in weight and in the test can be expressed by the correlation coefficient between the per cent change in weight and the change in the sum of the four blood sugar values. This coefficient was $+0.37 \pm 0.09$. The only possible basis for a

* The correlation in pounds and the

The data given below are based upon studies of patients who presented themselves during the past five years at the out-patient department of the Buffalo General Hospital. Among the preliminary studies was a glucose tolerance test which was carried out as follows: A specimen of venous blood was taken from the patient while he was in the post-absorptive state. He then ingested 100 grams of glucose dissolved in 250 cc of water. Thirty minutes, one hour and two hours after the glucose was ingested specimens of venous blood were drawn. Blood sugar concentrations were determined by Myers' and Bailey's (21) modification of the picric acid method of Lewis and Benedict (22). Patients were then seen by one of the authors (E C B) who prescribed the diet consisting of 80 grams of protein, 40 grams of fat and 40 grams of carbohydrate described in detail in the preceding article (1). The patients were seen at fortnightly intervals thereafter, and records were made of their weights and of changes in their symptoms. At each visit the diet was discussed and the patients were urged to carry out the treatment carefully. It is probable that the diet was not followed exactly, but the reduction in food intake must have been marked, for substantial losses in weight were shown by the majority of the patients who maintained their relationship with the clinic. When the weight had been reduced to an extent which seemed reasonably satisfactory, the glucose tolerance test was repeated.¹ The present article is based upon a study of 39 patients who were brought approximately to normal weight. No method of selection was used except that patients with diabetic symptoms were not studied. Otherwise, all patients presenting themselves at the clinic whose weight was reduced to an extent which the physician in charge (E C B) considered reasonably satisfactory, and upon whom a second tolerance was obtained after weight reduction, have been included.

Of the 39 patients all but 2 were women. Their

¹ Upon only 5 of the 39 patients were tests carried out between the initial one and the studies made after weight reduction. Two patients were studied twice and 3 once during the period of treatment. Since the therapy was continued for an average period of 350 days, it seems improbable that any differences noted between the two sets of tests could be attributed to adaptation to the procedure.

ages ranged from 25 to 72 years (only one was over 70) and averaged 44 years. Their initial weights averaged 200 pounds and ranged from 151 to 265 pounds. Only 2 weighed less than 160 pounds. In comparison with the figures given in Davenport's table (23), these patients averaged 52 per cent above normal weight. The individual weights ranged from 12 to 97 per cent above normal. Only 2 were less than 25 per cent above normal. The average time during which the patients took the diet before the tests were repeated was 350 days, with extreme values of 196 and 862 days. During this period they lost between 26 and 110 pounds apiece. The average weight lost was 58 pounds. At the end of the period their weights averaged 8 per cent above normal, with a range of from 10 per cent below to 25 per cent above that figure. Only 1 was more than 20 per cent and only 4 were over 15 per cent above normal.

Before proceeding to an analysis of the data obtained upon these patients, it seemed desirable to determine whether the results of the glucose tolerance tests made upon them can be regarded as approximately representative of those of a large group of obese subjects. Table I shows that

TABLE I
Results of glucose tolerance tests made upon patients when they entered the clinic

Blood specimens analyzed	Average values		Median values		Standard deviations	
	*233 cases	†39 cases	*233 cases	†39 cases	*233 cases	†39 cases
	milligrams per 100 cc.		milligrams per 100 cc.		milligrams per 100 cc.	
Specimens were taken before glucose was given	125±0.8	127±1.5	122±1.0	127±1.9	17±0.3	14±1.1
½ hour after glucose	182±1.4	184±4.6	179±1.7	183±5.8	32±1.0	28±2.1
1 hour after glucose	183±2.0	191±4.2	181±2.5	186±4.3	47±1.4	39±3.0
2 hours after glucose	149±1.9	155±3.7	143±2.4	154±4.6	43±1.3	34±2.6

* Under "233 cases" are the results of glucose tolerance tests made upon all of the obese patients who were studied when they first entered the clinic.

† Under "39 cases" are the first results obtained upon the patients who were also studied after their weights had been reduced approximately to normal.

they can be properly so considered, for the averages of the results upon these 39 patients are practically identical with those obtained upon 235 patients who were accepted for treatment by the obesity clinic.

These average initial figures were high but

are known to influence such tests in two distinct ways. Starvation (31) and prolonged undernutrition (32) frequently produce a lowering of the fasting blood sugar. On the other hand fasting (33) and the ingestion of diets low in carbohydrate (34) cause an increase in the rise of blood sugar which follows the ingestion of a test dose of glucose. To determine the significance of the changes shown in Table II it is therefore necessary to see whether the changes in the fasting blood sugar, in the rise which follows the ingestion of a test dose of glucose or in both are significant. The median change in the fasting blood sugar was 17 ± 1.6 mgm per 100 cc., all patients but 4 showed some decrease. The decrease in the total rise after glucose ingestion (the sum of the differences between the blood sugar concentration and the value found in the fasting blood) was 93 ± 9.4 mgm per 100 cc. all but 4 patients showed some decrease. The median decrease in the maximum rise above the fasting blood sugar level was 36 ± 5.1 mgm per 100 cc., only 6 patients failed to show this decrease. It is evident that a large proportion of the 39 patients showed decreases both in the fasting blood sugar and in the rise following the ingestion of the test dose of glucose, and that both changes were significant. Examination of the data available in other reports (6, 7, 8) shows that such findings are usual when reduction in the weight of obese subjects is brought about by dietary therapy.

It seemed desirable to determine whether these changes persisted when a diet more liberal than the one used in the therapy was ingested. The following experiment was devised to investigate this question. After the degree of weight reduction was considered fairly satisfactory some of the patients were urged to eat a more liberal diet and the amount of food prescribed was increased by the addition of 200 to 250 grams of carbohydrate. After they had taken these improved diets for about two months 25 of the patients returned to the hospital for another glucose tolerance test. They had gained very little weight during this period. The results of all the tests upon these 25 patients are given in Table III. It is evident that there was no apparent decrease in the tolerance following the increase in the diet. Instead there seems to have been a slight further

TABLE III
The apparent effect of weight reduction and diet upon the glucose tolerance tests of 25 patients

Blood specimens analyzed	Median values			Standard deviations		
	*Before weight reduction	†After weight reduction (low diet)	‡After weight reduction (liberal diet)	*Before weight reduction	†After weight reduction (low diet)	‡After weight reduction (liberal diet)
Specimens were taken before glucose was given	133 ± 2.6	112 ± 1.9	110 ± 1.9	16 ± 1.5	11 ± 1.0	11 ± 1.0
1 hour after glucose	153 ± 5.8	152 ± 4.0	153 ± 5.8	20 ± 2.7	27 ± 2.8	27 ± 2.8
1 hour after glucose	189 ± 4.4	180 ± 6.5	183 ± 4.0	23 ± 3.6	28 ± 3.8	28 ± 3.3
2 hours after glucose	184 ± 6.4	155 ± 4.5	173 ± 3.9	38 ± 5.0	25 ± 2.7	27 ± 3.2

These results were obtained upon 25 patients who were studied three times.

*Under 'before weight reduction' are the figures obtained upon these patients when they were first seen.

†Under 'after weight reduction—low diet' are the figures when they had been reduced approximately to normal weight while taking the diet first prescribed.

‡Under 'after weight reduction—liberal diet' are the results found when they had been upon a more liberal diet for between one and two months as described in the text.

Averages were also computed. They were not significantly different from the median values given and have therefore been omitted.

improvement. The median fall in the sum of the four blood sugar values was 34 ± 14 mgm. per 100 cc. This change was largely or wholly due to a change in the rise of blood sugar after the ingestion of glucose, for the median fall in the fasting blood sugar was 2 ± 2.4 mgm per 100 cc., and that of the decrease in the total rise after the ingestion of glucose was 26 ± 13.1 mgm per 100 cc. The latter change may correspond to the normal response shown by glucose tolerance tests when an increased amount of carbohydrate is ingested prior to the examination but it is probably so slight as to deserve no emphasis. The change in the fasting blood sugar certainly is of no significance.

It seems to the authors that only two explanations can be offered for the significant changes produced in the glucose tolerance tests of these 39 patients. The first is a direct effect of diet upon the test. It is possible that when diets low in carbohydrate are ingested for long periods of time they do not cause the usual increase in the blood sugar rise after a test dose of glucose but instead cause a decrease. This seems improbable, but not perhaps wholly impossible. On the basis of such an explanation for the findings an interpretation of the results obtained after the im-

calculation of the effect of diet in these studies was the rather unsatisfactory one based upon the length of time during which the diet was ingested. The correlation coefficient between the number of days on the diet and the total change in the glucose tolerance test was $+0.14 \pm 0.10$. It is clear that these calculations furnish no evidence that the diet has directly produced the improvement in the glucose tolerance test. They do suggest that this improvement may be associated with the decrease in weight.

The individual glucose tolerance curves obtained after treatment were also examined to determine whether many of them were markedly abnormal. In 30 of the 39 patients all of the blood sugar values in such a test were within the normal limits given by McCullagh and Johnston (24). In only 3, or 8 per cent, were the fasting blood sugar concentrations greater than 120 mgm per 100 cc. Only 3 patients showed high values in more than one of the four blood specimens. Of these 3 only 1 gave high values in all specimens. In the tests upon 4 of the patients a value which was slightly (5 mgm per 100 cc or less) below the normal was found in a single specimen. It is evident that the tests upon most of the patients after dietary therapy should be considered normal.

Not only the actual values found in the final tests, but also the differences between the initial and final tests were studied. Only 4 of the patients failed to show a decrease in the sum of the four blood sugar values. These 4 included the 3 patients referred to in the preceding paragraph, who had high blood sugar values in more than one specimen. An examination of the clinical histories of these 4 patients was made to determine whether any explanation could be found for the failure of these tests to respond during treatment as did those of 90 per cent of the subjects studied. The results of this examination were suggestive, but no entirely satisfactory explanation of the failure was found. Three of the 4 patients showed hypertension with retinal signs of arterial damage, findings often associated with abnormal glucose tolerance curves (25), but this clinical condition did not seem to be more marked in these 3 patients than in others in the series. One of these 3 patients was the only subject over 70 years old included, and her advanced

age may have directly affected the glucose tolerance test (26, 27). The fourth patient in the group was suffering from arthritis, and it has been repeatedly reported (28, 29, 30) that most of the patients with this disease have abnormal glucose tolerance curves. However, 3 other patients with arthritis showed an improvement in tolerance following the period of therapy, and it is probably not proper to place great emphasis upon the presence of this disease as an explanation of the failure in this instance.

Since most of the authors who have studied changes in the glucose tolerance test during weight reduction (6, 7, 8) have chosen as subjects persons with distinctly high initial tests, and since the patients discussed in the present paper were not selected in this way, the data were studied to see first, whether there was any correlation between the initial tests and the changes observed after treatment, and, second, whether the patients with tests which were approximately normal showed changes similar to those noted in the majority of the subjects. The correlation coefficient between the sum of the initial four blood sugar concentrations and the change in that value after treatment was $+0.32 \pm 0.10$, probably denoting a significant but not a very marked correlation. Of the 5 patients with all of the four initial blood sugar values within normal limits, 2 showed decreases which were probably within the limit of error of the technical method used, but 2 others showed changes which were greater than the average of the whole series of patients. One of these 5 patients had a concentration of sugar in the specimen drawn 0.5 hours after the ingestion of glucose which was slightly (5 mgm per cent) below the normal value given by McCullagh and Johnston. All the other values were within the rather wide normal range given by those authors. These results, while they show that the most marked improvement in the glucose tolerance occurs in patients with the most markedly abnormal initial values, show also that the response of practically all obese subjects to weight reduction through dietary therapy is probably essentially the same.

Before proceeding further with a discussion of the results, a somewhat detailed consideration of the known effect of diet upon the glucose tolerance test is necessary. Diets low in food value

normal glucose tolerance tests was high (87 per cent) but the degree of abnormality was not marked. After weight reduction had been produced by dietary therapy, 90 per cent of the patients showed some improvement in the test and only 23 per cent showed any abnormality demonstrable by the methods used. The improvement in tolerance appeared to be due to the weight reduction rather than directly to the diet, for (1) the change in the curve persisted when the amount of carbohydrate fed was increased (2) was of a type which could not readily be explained by the ingestion of diets low in food value and (3) paralleled roughly the changes in weight.

BIBLIOGRAPHY

- 1 Beck, E. C., and Hubbard, R. S., Study of obesity in outpatient clinic. *Am. J. Digest. Dis. and Nutrition*, 1934 1 250
- 2 Kisch, E. H., *Der Diabetes der Alternenden. Medizinische Klinik*, 1915 11, 164 Abstract *J. A. M. A.*, 1915 64 1038.
- 3 Paullin, J. E., and Sauls, H. C. Glucose tolerance test in obese. *Southern M. J.*, 1922, 15, 249
- 4 John, H. J., Summary of findings in 1100 glucose tolerance estimations. *Endocrinology* 1929 13, 388.
- 5 Ogilvie, R. F., Sugar tolerance in obese subjects: review of sixty five cases. *Quart. J. Med.*, 1935 4, 345
- 6 Rony, H. R., Observations on "prediabetes" *Endocrinology* 1937 21 195
- 7 Fetter, F., Durkin, J. K., and Duncan, G. G. Dietary versus insulin treatment of obese diabetic patient. *Am. J. M. Sc.* 1938 195, 781
- 8 Newburgh L. H., and Conn J. W., A new interpretation of hyperglycemia in obese middle-aged persons. *J. A. M. A.*, 1939 112 7
- 9 Herrmann, E. T., Glycosuria and glycaemic tolerance curve review. *Am. J. Clin. Path.*, 1932, 2 87
- 10 Myers G. B., and McKean R. M., The oral glucose tolerance test: review of the literature. *Am. J. Clin. Path.*, 1935 5 299
- 11 Peters J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry Vol. 1 Interpretations*. Williams and Wilkins Co., Baltimore, 1931 pp. 114 to 129
- 12 Lemox, W. G. Repeated blood sugar curves in non diabetic subjects. *J. Clin. Invest.* 1927 4 331
- 13 Joslin E. P., Prevention of diabetes mellitus. *J. A. M. A.*, 1921 76 79
- 14 Harris, S. Hyperinsulinism and dysinsulinism. *J. A. M. A.*, 1924 83, 729
- 15 Harris S., Hyperinsulinism and dysinsulinism (in sulogenic hypoglycemia) with chronological review of cases reported in United States and Canada. *Endocrinology* 1932 16, 29
- 16 Winans, H. M., Chronic hypoglycemia. *South. M. J.* 1930 23 402.
- 17 Müsser J. H., and Wright, D. O., Hypertension obesity and hyperglycemia. *J. A. M. A.*, 1933 101, 420
- 18 Tyner J. D. Pre-diabetic state its relation to obesity and diabetic heredity. *Am. J. M. Sc.* 1933 185, 704
- 19 Tyner J. D., Pre-diabetic state its treatment by low carbohydrate diet and reduction in weight. *J. Lab and Clin. Med.*, 1931-32, 17, 456
- 20 Brill I. C., Effect of a normal meal upon blood sugar level in health and disease. *J. Lab and Clin. Med.*, 1923 8 727
- 21 Myers V. C., and Bailey C. V. Lewis and Benedict method for estimation of blood sugar with some observations obtained in disease. *J. Biol. Chem.*, 1916, 24 147
- 22 Lewis, R. C. and Benedict, S. R. Method for estimation of sugar in small quantities of blood. *J. Biol. Chem.* 1915 20 61
- 23 Davenport, C. B., Carnegie Institute of Washington. Publication Number 329 1923 pp 19 20 27, 169 to 174
- 24 McCullagh, E. P. and Johnston C. R. K. Manipulation of glucose tolerance by diet. *Am. J. M. Sc.*, 1938 195 773.
- 25 Kerpola W., Zur Kenntnis der essentiellen Hypertonie. *Acta Med. Scandinav.* 1922-23, 57 515
- 26 Punschel A., Der Blutzucker im höheren Lebensalter und besonderer Berücksichtigung der alimentären Hyperglykämie. *Ztschr. f. klin. Med.*, 1923 96, 253
- 27 Spence, J. C. Sugar tolerance, with special reference to variations found at different ages. *Quart. J. Med.*, 1921 14 314
- 28 Pemberton, R., and Foster G. L., Studies on arthritis in the army based on four hundred cases. III. Studies on the nitrogen urea carbon dioxide combining power calcium, total fat and cholesterol of the fasting blood, renal function, blood sugar and sugar tolerance. *Arch. Int. Med.* 1920 25 243
- 29 Holsti O., Alimentäre Hyperglykämie bei Arthritis. *Acta Med. Scandinav.*, 1922, Supplement III, 137
- 30 Fletcher A. A., Diabetic treatment of chronic arthritis and its relationship to sugar tolerance. *Arch. Int. Med.*, 1922, 30 106
- 31 Lennox, W. G., O'Connor M., and Bellinger M., Chemical changes in the blood during fasting in the human subject. *Arch. Int. Med.* 1926 38 553
- 32 Holst, J. E. Untersuchungen über leichte Glykosen. *Acta Med. Scandinav.*, 1922-23 57 188.
- 33 Sweeney J. S., Dietary factors that influence the dextrose tolerance test preliminary study. *Arch. Int. Med.*, 1927 40 818.
- 34 Staub H., Untersuchungen über den Zuckerstoffwechsel des Menschen. *Ztschr. f. klin. Med.*, 1922 93, 123
- 35 Sherrill J. W., Abstract of Discussion. *A.*, 1939 112, 10.

provement in the diet is very difficult. Statistical analysis afforded no evidence favoring such an explanation. A second possible cause of the results is some direct or indirect effect of weight reduction upon the metabolism of glucose. Such an explanation avoids the difficulty in interpreting the change in the rise of blood sugar after the test dose of glucose. It affords an adequate explanation of the persistence of the improvement when the more liberal diets were ingested. It is supported to some extent by the existence of some degree of parallelism between the change in weight and the improvement in the glucose tolerance. The authors believe that some effect of the change in weight upon the glucose tolerance is probably the correct explanation of the findings presented.^a

It is difficult to decide by what mechanism the change in weight may have affected the glucose tolerance. Certain theories have been advanced by investigators who have discussed the rather extreme cases which sometimes simulate diabetes mellitus. Sherrill (35) has suggested that there may be a deficiency of insulin production in obese glycosuric patients. Since Newburgh and Conn (8) showed that such subjects burn glucose as readily as do normal persons, this explanation cannot be considered wholly satisfactory. However, it seems possible that the amount of insulin formed by these subjects does not completely meet the needs of the patients when their weight is maximal but does so after the weight has been reduced. Newburgh and Conn (8) believe that the deposition of fat in the liver prevents that organ from removing glucose from the circulating blood as readily as it normally does. This theory seems adequate to account for the initial abnormality of the blood sugar rise after glucose ingestion, and for the improvement in that rise after weight reduction. It is not quite so clear how the frequent high values of the fasting blood

^a Further evidence favoring the existence of a relationship between variations in weight and in the glucose tolerance test is afforded by the results upon the case of recurrent obesity reported by Newburgh and Conn (8). This patient showed abnormal glucose tolerance curves on two occasions when she was overweight, and normal tests upon two occasions when her weight had been brought to normal by dietary therapy. One of the authors of the present communication (E. C. B.) has 2 patients under observation at the present time who are apparently showing similar variations, but the studies upon them have not been completed.

sugar of obese subjects can be explained by it. This criticism is probably not an insurmountable one, but it does make the authors hesitate to accept the thesis without reservations. It seems best merely to state that in obese subjects the metabolism of glucose is very frequently somewhat different from that of the average normal person, and that when the weight is reduced the degree of this abnormality is very often decreased.

A comparison of previous studies with the present one shows one thing quite clearly. There was no significant qualitative difference between the responses in the group of experiments reported here and those which have been described by other investigators. The quantitative differences, however, were marked. These differences were largely or wholly due to the methods used in selecting the patients. In work previously presented, only patients with markedly abnormal glucose tolerance curves, frequently simulating clinical diabetes (8), were intensively studied. The material for the present study was chosen differently. The admitting office of the outpatient department did not refer patients thought to have diabetes to the obesity clinic. When obese patients with symptoms of diabetes were admitted to the clinic they were referred elsewhere as soon as their condition was recognized, although not more than 3 subjects were rejected during the period covered by the present paper. Such methods of selection must to some extent have weighted the series in favor of patients with little impairment of carbohydrate metabolism. The authors believe the results obtained demonstrate the presence of a mild impairment of carbohydrate metabolism in these patients and a response to therapy which was essentially the same as that shown by subjects with more marked degrees of abnormality. They believe that the following conclusions are justified. Most, although probably not all (6, 14, 15, 16) patients with obesity, show some abnormality in the tolerance for glucose. This abnormality can usually be markedly improved by weight reduction through dietary therapy.

CONCLUSIONS

A study of 39 obese subjects who were selected in such a way as to exclude patients with marked abnormalities in glucose metabolism is presented. The proportion of these patients who showed ab-

THE RELATIONSHIP BETWEEN THE ERYTHROCYTE SEDIMENTATION RATE AND THE PLASMA PROTEINS^{1 2}

By MARIAN W ROPES, ELSIE ROSSMEISL AND WALTER BAUER

(From the Medical Clinic of the Massachusetts General Hospital the Department of Medicine Harvard Medical School and the Massachusetts Department of Public Health Boston)

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From the time of the early Greek physicians many observers noted a relationship between the sedimentation rate of the red cells and the concentration of fibrinogen or "phlegma" in the blood. However, no detailed studies were made until 1918 when Fahraeus (5) studied the sedimentation rate in pregnancy and concluded that the increase in the rate was due to a lowering of the electric charge on the red cells. Since that time interest in the subject has been renewed. Numerous investigators have studied the variations in sedimentation rate that occur in disease and a few workers have attempted to determine also the factors underlying these variations. As a result of these investigations of the past twenty years, the sedimentation rate has become of definite clinical value, but there is no general agreement as to the factors involved in the aggregation and sedimentation of the red cells.

The majority of investigators have corroborated the long standing impression that there is a suggestive relationship between the concentration of fibrinogen and the sedimentation rate. In fact, despite occasional marked exceptions to an exact linear relationship between the fibrinogen and the sedimentation rate, the majority of recent workers have concluded that the concentration of fibrinogen determines the sedimentation rate (1, 2, 4, 8, 10, 14, 16). The occurrence of occasional marked inconsistencies, however, has led a few workers to conclude that the relationship between fibrinogen and sedimentation rate is not one of cause and effect (9, 12, 13).

Various other constituents of the blood, notably globulin and lipoids, have been suggested as regulating factors in the sedimentation rate of the red cells. The majority of investigators agree

that blood samples with high concentrations of globulin have high sedimentation rates.

In the course of our studies of the sedimentation rates and plasma protein fractions in arthritis, we occasionally obtained marked inconsistencies in the relationship between plasma proteins and sedimentation rates, in contrast with the majority of the findings reported in the literature. Because of these findings and the lack of agreement as to the correlation between the plasma proteins and the sedimentation rate, which is evident from a survey of the literature, we undertook a more detailed study of the relationship.

The present investigation includes chiefly studies in various types of arthritis, but it also includes a few studies in other diseases which produce abnormal rates, such as myelomatosis, carcinoma of lung, malignant lymphoma of Hodgkin's type, acute lupus erythematosus disseminatus, poikiloderma atrophicans vasculare, lymphogranuloma inguinale and nutritional edema. The results are of special significance since they cover both the sudden and marked changes in sedimentation rates and in concentration of proteins that occur in acute infections such as gonorrheal arthritis, and the more gradual changes in chronic infections such as rheumatoid arthritis. In conditions in which the type of reaction is so different, the chemical changes produced by the infection would be expected to be different. Whatever the stimulating agent may be which is produced by the infection and which leads to changes in the proteins and the sedimentation rate, it acts more slowly and over a longer period of time in a chronic infection like rheumatoid arthritis. This makes it possible to study the relationship of the variations in rates to the variations in the concentrations of proteins more accurately than in the case of acute infections. For, in acute infections, the changes are so sudden and of such magnitude that a superficial relationship may be apparent but it may be impossible to follow the changes closely enough to

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² The expenses of this investigation were defrayed in large part by the Commonwealth Fund.

of gonorrheal arthritis 22 from 16 cases of rheumatoid arthritis, 7 from 5 cases of myelomatosis 1 from 1 case of nutritional edema, 1 from 1 case of carcinoma of the lung with pulmonary osteoarthropathy, 1 from 1 case of Charcot's joint, 1 from 1 case of malignant lymphoma of Hodgkin's type, 3 from 3 cases of arthritis of unknown origin 1 from 1 case of degenerative joint disease, 1 from 1 case of acute lupus erythematosus disseminatus 1 from 1 case of poikiloderma atrophicans vasculare, 2 from 2 cases of lymphogranuloma inguinale, and 1 from 1 case of chronic glomerular nephritis

The results given in Table I show that, in general, blood samples with high sedimentation rates

have high concentrations of fibrinogen or of globulin, in accordance with the findings of numerous other workers. More detailed analysis of the figures, however, shows that there are many variations of greater or lesser degree in the relationship between the rates and the concentrations of the protein fractions

Comparison of the sedimentation rates with the fibrinogen concentrations (see Figure 1) shows no clear-cut linear relationship such as that found by Giligan and Ernestine (8), and by Ham and Curtis (10). There is only a suggestion of a linear relationship and at least one-third of the values are not consistent with such a relationship. A few of the inconsistencies may be explained, in part at

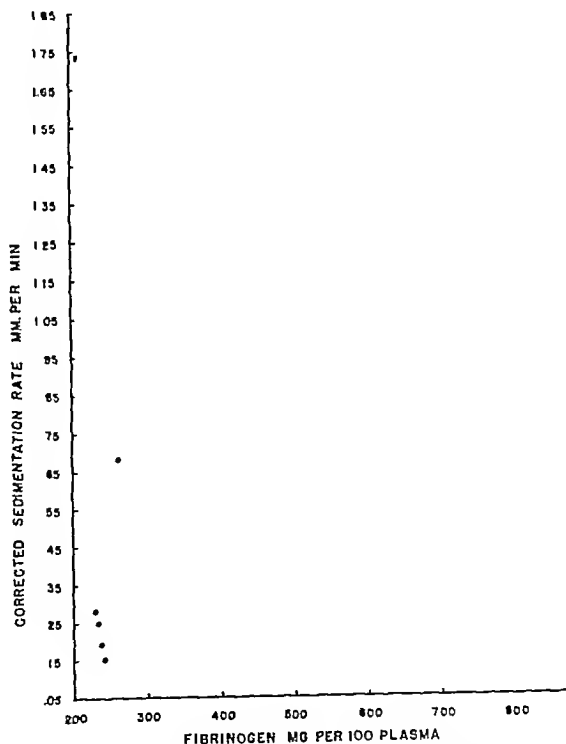


FIG. 1 CORRELATION BETWEEN THE CORRECTED SEDIMENTATION RATE AND THE PLASMA FIBRINOGEN

determine the order of occurrence and draw conclusions as to the causal relationship

METHODS

The subjects in this series included patients in the hospital and ambulatory patients seen in the clinic. The patients were fasting with the exception of the few cases indicated in the tables. The blood samples were withdrawn without stasis from the median basilic vein. Heparin (product of Hynson, Westcott and Dunning), in a concentration of 4 mgm for approximately 4 cc. of blood, was used as an anticoagulant in the blood samples for the determination of sedimentation rates. Potassium oxalate, in a concentration of 2 mgm. per cc., was used as an anticoagulant in the samples for the fibrinogen determinations. No anticoagulant was used in the blood samples for the total protein and albumin determinations.

The erythrocyte sedimentation rates were determined by the method of Rourke and Ernstene (15), and corrected for hematocrit readings by the use of their correction chart. This method, by virtue of the length and diameter of the tube and by the determination of the rate during the period of most rapid fall, has been shown to avoid errors introduced in other methods from packing, counter flow, and inclusion in the rate of varying proportions of the initial slow period of aggregation and subsequent rapid period of settling (10). Furthermore, this method is the one which was found by Ham and Curtis (10) to give a higher statistical correlation with the fibrinogen content than the Wintrobe, Westergren, Cutler, or Linzenmeier methods. It was with the use of this method also that Gilligan and Ernstene (8) and Ernstene (4) obtained an approximately linear correlation between the sedimentation rate and the fibrinogen content.

The total protein was obtained by determination of the total nitrogen by a modified macro-Kjeldahl. The difference between the total nitrogen and the nonprotein nitrogen, determined by the method of Folin and Wu (7), was multiplied by the factor 6.25 to give the total protein. The albumin content was determined by the method of Howe (11), using 22.5 per cent sodium sulphate. The fibrinogen content was determined by precipitation as fibrin by the method of Cullen and Van Slyke (3) and determination of the nitrogen by digestion and nesslerization.

The following values have been accepted as the upper limits of normal

Protein	8.0	grams per 100 cc.
Albumin	5.5	grams per 100 cc.
Globulin	3.0	grams per 100 cc.
Fibrinogen	.350	grams per 100 cc.

RESULTS

The blood samples included in this investigation were from the following sources: 46 from 8 cases

TABLE I

Relationship of sedimentation rate to plasma proteins

Case number*	Corrected sedimentation rate	Total protein	Albumin	Globulin	Fibrinogen
	mm per minute	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc.
I 1	1.54	7.77	4.41	3.36	0.575
2	1.55	7.85	4.85	3.00	0.469
II	0.37	7.75	5.01	2.73	0.288
III 1	1.64	8.63	4.74	3.89	0.652
2	1.12	9.17	4.95	4.22	0.614
IV 1	1.95	10.40	3.30	7.10	
2	1.58	9.45	3.41	6.04	
3	1.36	8.59	3.64	4.94	0.631
V	1.24	7.82	4.92	2.91	0.366
VI	0.50	7.10	4.42	2.68	0.293
VII	1.40	8.19	4.95	3.25	0.625
VIII	1.27	8.74	4.91	3.83	0.375
IX	0.98	8.28	4.59	3.68	0.458
X 1	0.25	7.80	5.00	2.81	0.234
2	0.34	7.45	5.24	2.21	0.256
XI	0.37	7.12	4.94	2.14	0.308
XII	0.53	7.35	4.24	3.12	0.225
XIII	0.92	7.71	4.76	2.95	0.338
XIV 1	0.30	7.70	5.25	2.44	0.292
2	0.29	8.00	5.06	2.94	0.269
XV	0.80	7.57	4.96	2.61	0.658
XVI	0.12	7.07	4.97	2.10	0.236
XVII	1.43	7.06	3.84	3.22	0.511
XVIII	1.83	8.30	4.66	3.64	0.850
XIX 1	1.55	7.91	4.87	3.04	0.823
2	1.69	6.81	4.36	2.45	0.726
XX	1.63	8.10	4.13	3.97	0.568
XXI	1.96	7.98	4.07	3.91	0.972
XXII	0.68	7.66	4.51	3.15	0.264
XXIII 1	1.14	10.53	2.92	7.61	
2	1.19	10.36	2.84	7.52	0.306
3	0.75	11.77	3.35	8.43	0.511
XXIV†	1.77	12.60	3.64	8.96	0.418
XXV	1.42	8.39	3.83	4.57	0.313
XXVI†	1.80	8.57	2.81	5.76	0.483
XXVII	0.12	3.90	2.84	1.06	
XXVIII	1.16	7.35	4.64	2.71	0.420
XXIX	0.74	6.72	4.42	2.30	0.335
XXX	1.52	6.25	4.00	2.25	0.628
XXXI	0.90	7.62	5.22	2.40	0.504
XXXII	0.64	6.47	4.45	2.02	0.373
XXXIII	1.86	7.68	3.77	3.91	0.940
XXXIV	1.01	4.18	2.40	1.78	0.436
XXXV	0.15	7.10	4.62	2.49	0.289
XXXVI	1.40	7.48	4.37	3.12	0.366
XXXVII	0.52	7.84	5.22	2.62	0.315
XXXVIII	1.22	6.15	2.99	3.16	0.631
XXXIX	1.46	7.87	4.90	2.98	0.609

* Diagnoses: Cases I through XVI—rheumatoid arthritis, Cases XVII through XXI—gonorrheal arthritis, Cases XXII through XXVI—myelomatosis, Case XXVII—nutritional edema, Cases XXVIII, XXXI and XXXVI—arthritis of unknown origin, Case XXIX—Charcot's joint, Case XXX—carcinoma of lung with hypertrophic pulmonary osteoarthropathy, Case XXXII—degenerative joint disease, Case XXXIII—malignant lymphoma of the Hodgkin's type, Case XXXIV—chronic glomerular nephritis, Case XXXV—lupus erythematosus disseminatus, Case XXXVII—poikiloderma atrophicum vasculare, Cases XXXVIII and XXXIX—lymphogranuloma inguinale

† Patient not fasting

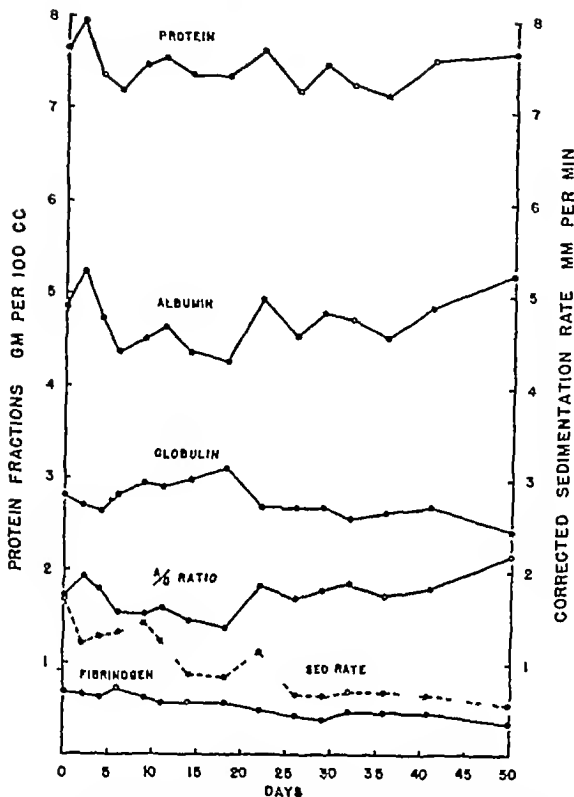


FIG. 2. THE VARIATIONS IN THE CORRECTED SEDIMENTATION RATE AND PLASMA PROTEIN FRACTIONS IN A CASE OF GONORRHEAL ARTHRITIS TREATED WITH SULFANILAMIDE

mentation rate with a 5 per cent rise in fibrinogen, and then a 20 per cent drop in fibrinogen with no change in sedimentation rate. Similarly in Case 33, a 30 per cent drop occurred in sedimentation rate with no change in the fibrinogen concentration.

The fact that the curves for fibrinogen, globulin and sedimentation rate show moderate correlation but are not superimposable indicates that the changes represent a direct response of all factors (fibrinogen, globulin and sedimentation rate) to the stimulating agent, rather than a response of one indirectly because of a change in one of the other factors.

Further experiments were undertaken to prove that changes in sedimentation rates are not necessarily associated with changes in the concentrations of any of the plasma proteins. The pH of one portion of a sample of heparinized blood was changed by addition of acid or alkali without significant alteration in the concentration of the proteins. The sedimentation rates of the two portions were then determined. The determinations of sedimentation rates were not done on blood

least, by abnormally high globulin concentrations. However, in some of the bloods that show higher sedimentation rates than would be expected from the fibrinogen content, the concentration of globulin is normal.

Comparison of the sedimentation rates with the total protein, albumin, and globulin concentrations and with the albumin/globulin ratios shows no linear relationship. Blood samples with high globulin contents tend to have elevated sedimentation rates, but there are even more inconsistencies than in the relation of fibrinogen concentration to sedimentation rate.

Analysis of individual blood samples shows clearly the lack of absolute correlation between the concentrations of fibrinogen and globulin and the rate of sedimentation of the red blood cells. The most marked variations are found in Case 5 (a girl of 15 who had had rheumatoid arthritis for 8 months and had had a recent acute exacerbation of joint symptoms). The corrected sedimentation rate rose to 1.24 mm per minute, although the concentration of fibrinogen and of globulin remained at the upper limits of normal. Furthermore the results in Case 22 indicate that increased concentrations of fibrinogen or of globulin do not necessarily increase the sedimentation rate. With progress of the disease (myelomatosis) the fibrinogen increased from 0.306 to 0.511 gram per 100 cc. and the globulin from 7.5 to 8.43 grams per 100 cc., but the sedimentation rate dropped from 1.19 to 0.75 mm per minute. In this case a 67 per cent rise in fibrinogen and a 12 per cent rise in globulin were associated with a 27 per cent fall in sedimentation rate.

Further evidence of lack of absolute correlation between the sedimentation rates and the fibrinogen and globulin concentrations is found in the following cases: Case 3 in which a 32 per cent drop in the sedimentation rate was associated with a slight fall (6 per cent) in the fibrinogen and a slight rise (4 per cent) in the globulin, Case 6 which showed a slight elevation in sedimentation rate with normal globulin and fibrinogen concentrations, Case 10 which showed a normal sedimentation rate with a fibrinogen concentration of 0.469 gram per 100 cc.

In order to study more thoroughly the correlation between plasma proteins and sedimentation rates, and to determine, if possible, whether the changes in protein precede or follow the alterations in

sedimentation rate, successive determinations were made in 3 cases of gonorrheal arthritis during periods of clinical change. (The patients were receiving sulfanilamide.) The results are shown in Table II and Figure 2. The curves for fibrinogen

TABLE II
Relationship of sedimentation rate to plasma proteins

Case number*	Corrected sedimentation rate	Total protein	Albumin	Globulin	Fibrinogen
	mm per minute	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc
XL 1†	1.73	7.67	4.85	2.83	0.688
2	1.19	7.96	5.25	2.71	0.638
3†	1.28	7.38	4.74	2.64	0.625
4	1.34	7.22	4.38	2.84	0.721
5	1.43	7.48	4.52	2.96	0.610
6	1.23	7.57	4.65	2.91	0.558
7	0.86	7.39	4.39	3.00	0.559
8†	0.83	7.39	4.27	3.12	0.564
9	1.12	7.66	4.97	2.69	0.494
10	0.65	7.24	4.55	2.69	0.415
11	0.62	7.50	4.81	2.69	0.375
12	0.73	7.29	4.75	2.55	0.469
13	0.72	7.19	4.55	2.63	0.446
14	0.68	7.56	4.86	2.70	0.440
15	0.53	7.65	5.23	2.42	0.317
XLI 1	1.75	7.41	3.98	3.43	0.792
2	1.35	6.95	3.91	3.05	0.834
3	1.36	6.90	3.87	3.04	0.662
4	0.43	6.78	3.91	2.87	0.458
5	0.25	6.51	3.78	2.72	0.375
6	0.18	6.20	3.77	2.43	0.277
7	0.28	6.83	4.59	2.24	0.230
XLII 1	0.75	7.24	4.23	3.01	0.497
2	1.12	7.09	4.51	2.57	0.580
3	0.89	7.07	4.30	2.77	0.521
4	0.75	7.11	4.57	2.54	0.513
5	0.68	7.03	4.41	2.62	0.527
6	0.72	7.00	4.42	2.58	0.479
7	0.71	7.24	4.64	2.60	0.521
8	0.67	7.31	4.58	2.72	0.500
9	0.65	7.10	4.35	2.75	0.471
10	0.43	6.91	4.52	2.39	0.447
11	0.52	6.82	4.45	2.36	0.361
12	0.61	6.87	4.49	2.39	0.399
13	0.42	6.77	4.55	2.22	0.356
14	0.43	7.13	4.56	2.57	0.348
15	0.38	7.24	4.78	2.47	0.359
16	0.68	6.89	4.73	2.16	0.418
17	0.37	6.57	4.35	2.22	0.383
18	0.30	6.80	4.70	2.11	0.341

* Diagnosis in these 3 cases gonorrheal arthritis

† Patients not fasting

concentration and sedimentation rate show the same general trend, but they are not superimposable. Changes in sedimentation rate did not consistently precede or accompany changes in either fibrinogen or globulin. For example, in Case 34 there was first a 26 per cent drop in sedi-

diseases Coburn and Kapp (2), in their *in vitro* experiments, found that the sedimentation rates with added protein were never so high as those of the untreated sera with the same protein concentrations

A concept with which all of the findings are consistent is that variations in sedimentation rate are due to variations in the physical state of the plasma colloids, with consequent changes in the electric charges on the proteins and red cells. With this hypothesis it is possible to explain the usual relationship between the concentration of fibrinogen and the sedimentation rate and also to understand the inconsistencies found by most workers. This theory also explains why so many factors have been found to influence the sedimentation rate.

The above concept is in accord with the original theory of Fåhræus (5). He concluded that variations in sedimentation rate were due to changes in the magnitude of the electric charge on the red cells.

It is important to emphasize the fact that the results indicate that the stimulating agents produced in disease cause a change in the colloidal state of the plasma and a resulting increase in the sedimentation rate. They may coincidentally increase the fibrinogen or globulin and any such change in the concentration of individual colloids naturally affects the colloidal state of the plasma. However, our results and those of other workers prove that the change in colloidal state and the resulting increase in rate may occur without any change in the concentration of fibrinogen or globulin. In other words, the agents may work partly through an increase in fibrinogen or globulin, or they may change the colloidal state without any change in the concentration of the proteins.

SUMMARY

1 The erythrocyte sedimentation rates and the plasma protein fractions were determined in 89 blood samples from various diseases.

2 No absolute correlation was found between the sedimentation rate and any of the plasma-protein fractions. At least one-third of the findings were not consistent with a linear relationship between the fibrinogen concentration and the sedimentation rate.

3 In successive determinations during periods of clinical change, alterations in sedimentation rate did not consistently precede or accompany changes in either fibrinogen or globulin. In addition it has been shown that marked changes in the sedimentation rate can be produced without any alteration in the concentration of plasma proteins.

4 The only concept which explains all of the findings is that variations in sedimentation rates are due to variations in the colloidal state of the plasma with consequent changes in the electric charges on the proteins and red cells. Variations in the concentration of fibrinogen, globulin and other constituents affect the rate through their effect on the colloidal state of the plasma.

BIBLIOGRAPHY

- 1 Bendien, W. M., and Snapper, I., Zusammenhang zwischen der Senkungsgeschwindigkeit der roten Blutkörperchen und dem Eiweisspektrum. *Biochem. Ztschr.*, 1931, 14, 235.
- 2 Coburn, A. F., and Kapp, E. M., Observations on development of high blood sedimentation rate in rheumatic carditis. *J. Clin. Invest.*, 1936, 15, 715.
- 3 Cullen, G. E., and Van Slyke, D. D., Determination of fibrin, globulin and albumin nitrogen of blood plasma. *J. Biol. Chem.*, 1920, 41, 587.
- 4 Ernstene, A. C., Erythrocyte sedimentation, plasma fibrinogen and leukocytosis as indices of rheumatic infection. *Am. J. Med. Sc.*, 1930, 12, 180.
- 5 Fåhræus, R., Ueber die Ursachen der verminderten Suspensionsstabilität der Blutkörperchen während der Schwangerschaft (Vorläufige Mitteilung). *Biochem. Ztschr.*, 1918, 89, 355.
- 6 Fåhræus, R., Suspension stability of blood. *Physiol. Rev.*, 1929, 9, 241.
- 7 Folin, O., and Wu, H., System of blood analysis. *J. Biol. Chem.*, 1919, 38, 81.
- 8 Gilligan, D. R., and Ernstene, A. C., Relationship between erythrocyte sedimentation rate and fibrinogen content of plasma. *Am. J. Med. Sc.*, 1934, 187, 552.
- 9 Greisheimer, E. M., Johnson, O. H., and Ryan, M., Relationship between sedimentation index and fibrin content in relatively normal individuals. *Am. J. Med. Sc.*, 1929, 177, 816.
- 10 Ham, T. H., and Curtis, F. C., Sedimentation rate of erythrocytes: influence of technical, erythrocytic and plasma factors and quantitative comparison of five commonly used sedimentation methods. *Medicine*, 1938, 17, 447.
- 11 Howe, P. E., The determination of proteins in blood. A micro-method. *J. Biol. Chem.*, 1921, 49, 109.
- 12 Jones, L. R., Plasma proteins: red-cell sedimentation and serum lability of blood in tuberculosis. *Am. Rev. Tuberc.*, 1931, 23, 325.

mination was the significant value rather than the pH of the blood as drawn. Colorimetric determinations of pH with various indicators were made. It was found possible to change the pH approximately 0.5 in either direction without causing clotting or hemolysis. When the pH was altered by acetic acid there were no significant changes in the corrected sedimentation rates (Table III). The uncorrected rates decreased but

TABLE III
*Effect of acetic acid **

Case number	Corrected sedimentation rate	Hematocrit	Approximate pH
	<i>mm per minute</i>	<i>per cent</i>	
I a†	0.99	42.0	7.8
b†	0.89	43.0	7.4
II a	1.01	42.0	7.8
b	0.99	44.0	7.6
III a	1.24	42.0	7.8
b	1.17	43.5	7.4
IV a	1.21	34.5	7.8
b	1.18	40.0	7.0
V a	1.30	41.0	7.8
b	1.39	43.5	7.4
VI a	0.76	36.5	7.6
b	0.69	40.0	7.0
VII a	0.65	46.0	
b	0.72	48.5	

* Acetic acid concentration approximately 0.01 N

† a indicates original sample, b indicates sample to which acid has been added

there was an associated increase in the hematocrits. By adding solid sodium carbonate to a concentration of 0.1 or 0.2 per cent, there were marked changes in the corrected rates, a decrease in 3 cases and an increase in 1 case. The uncorrected rates were approximately equal in 2 cases but there was a coincident change in the hematocrits so that the corrected rates were very different (Table IV). Even more conclusive results were obtained when sodium hydroxide was added. The uncorrected rates and the hematocrits both decreased so that the corrected rates in the alkaline samples were much lower than those of the original samples. Thus, marked changes in sedimentation rates were produced without any change in the concentration of the proteins.

TABLE IV
*Effect of alkali **

Case number	Corrected sedimentation rate	Hematocrit	Approximate pH
	<i>mm per minute</i>	<i>per cent</i>	
VIII a†	0.74	34.0	7.5
b†	1.68	30.5	8.0
IX a	0.70	41.5	7.5
b	0.48	37.5	8.0
X a	1.98	39.0	7.5
b	0.45	31.5	8.0
XI a	0.66	40.5	7.5
b	0.35	30.0	8.0+
XII a	0.69	36.0	7.5
b	0.30	32.0	8.0
XIII a	1.91	39.0	7.5
b	1.50	35.5	8.0
XIV a	1.45	34.5	7.5
b	1.08	31.5	8.0
XV a	1.46	44.5	7.5
b	1.86	40.0	8.0
XVI a	1.25	41.0	7.5
b	0.78	37.5	8.0
XVII a	0.89	38.0	7.5
b	0.49	33.5	8.0

* Sodium carbonate (0.1 per cent) in Cases VIII and IX, sodium carbonate (0.2 per cent) in Cases X and XI, sodium hydroxide (approximately 0.01 N) in Cases XII to XVII

† a indicates original sample, b indicates sample to which alkali has been added

DISCUSSION

The numerous instances of lack of correlation between the erythrocyte sedimentation rate and the concentration of any of the plasma-protein fractions make it seem unlikely that an exact causal relationship exists.

In vitro experiments have proved that an increased concentration of fibrinogen or of globulin in any individual plasma does increase the sedimentation rate of the red cells in that plasma (2, 6, 14, 18). Fibrinogen has been found to have a greater effect than globulin. Albumin, on the other hand, has been found to have no effect or to decrease the rate. Such *in vitro* results, however, do not prove that increased concentrations of fibrinogen and globulin always cause increased rates *in vivo*, or that increases in these substances are the causes of the increased rates found in various

SPECIFIC VOLUME OF PLASMA AND SERUM PROTEINS IN PREGNANT AND IN PARTURIENT WOMEN AND THEIR NEWBORN CHILDREN AS DERIVED FROM VISCOSITY MEASUREMENTS

By FRED W. OBERST¹

(From the Department of Obstetrics and Gynecology, State University of Iowa, Iowa City, Iowa)

(Received for publication August 14, 1939)

Kunitz (1) reviewed the derivation of a formula developed by Einstein (2) indicating that viscosity is a function of the volume fraction of a dispersed substance, and suggested a modified empirical equation that was found to be more applicable to a large variety of solutions. The complete equation is

$$\eta = \frac{1 + 0.5\varphi}{(1 - \varphi)^2},$$

where η is the relative viscosity, i.e., the ratio of the absolute viscosity of the suspension to that of the pure solvent. The symbol φ stands for the volume fraction occupied by the dispersed substance or solute expressed either in percentage or in cubic centimeters (cc.) per 100 cc. of solution. Hence, φ divided by the weight of the solute per 100 cc. of solution represents the specific volume of the solute. This should be constant for various concentrations of a solute or a suspension unless the solute or suspension is hydrated or solvated to an extent varying with the concentration.

On expansion the equation becomes

$$\eta = 1 + 4.5\varphi + 12\varphi^2 + 25\varphi^3$$

When φ is very small, the equation becomes

$$\eta = 1 + 4.5\varphi$$

and is identical with that derived by Hatschek (3) for the viscosity of suspensions.

Kunitz advanced experimental data to demonstrate that the expanded equation may be applied to practically all solutions or suspensions where the solute or particles are large in comparison with the molecules of the solvent. The values for the specific volumes of various concentrations of sugar and sulphur suspensions, as calculated by this

equation, are constant and agree with the actual specific volume in the dry state. On the other hand, the values for the specific volumes of solutions of such substances as glycogen, casein, and rubber as obtained from viscosity measurements, are much higher than those recorded for the dry state, indicating that these substances are intimately associated with a portion of their solvents.

Viscosity measurements and protein determinations have been made on blood plasma and serum to calculate the specific volume of the respective proteins in terms of the expanded equation.

METHODS AND PROCEDURE

The viscosities of serum and plasma were determined at $25^\circ \pm 0.02^\circ \text{C}$ by means of a Poiseuille viscometer as modified by Ostwald, using 2 cc. portions. The relative viscosity (η) was obtained by dividing the time t_1 for serum or plasma by the time t_2 for isotonic (0.9 per cent) sodium chloride. When either the plasma or serum was diluted with a diluting fluid the corresponding value of t_2 was used. No correction was made for density of the serum or plasma and the diluting fluid, since this factor would be nearly one, and relatively constant.

The total nitrogen of the serum was determined by the micro-Kjeldahl method (4) and the non-protein nitrogen by the procedure of Folin and Wu (5). The protein nitrogen as obtained by subtraction was multiplied by the usual conversion factor, 6.25, to obtain the total protein percentage. Plasma protein was calculated by adding to the serum protein the value for fibrin, as determined by the method of Chandler (6).

To determine φ from the expanded equation when η is given, one must first plot on graph paper a curve similar to that given by Kunitz (1) on page 717. The values of φ for various values of η may then be read directly from the curve.

¹ Now with the U. S. Public Health Service Hospital, Lexington, Kentucky.

- 13 Moen, J K., and Reimann, H A., Plasma protein changes and suspension stability of blood in lobar pneumonia. *J Clin Invest.*, 1933, 12, 589
- 14 Oakley, W, Erythrocyte sedimentation and plasma fibrinogen *Lancet*, 1938, 1, 312
- 15 Rourke, M D, and Ernstene, A C, Method for correcting erythrocyte sedimentation rate for variations in cell volume percentage of blood *J Clin. Invest.*, 1930, 8, 545
- 16 Westergren, A, Theorell, H, and Widström, G, Plasmaeiweiss, Blutlipide, Erythrocyten und Senkungsreaktion *Ztschr f d ges exper Med.*, 1931, 75, 668
- 17 Yardumian, K., Physicochemical factors influencing red cell sedimentation rate. *Am. J Clin Path.*, 1937, 7, 105
- 18 von Zarday, I, and von Farkas, G, Quantitative Beziehungen zwischen Plasmaeiweissfraktionen und Blutsenkung *Ztschr f d ges exper Med*, 1931, 78, 367

TABLE II—C

The effect on the specific volume of the protein as determined by viscosity measurements of diluting serum with phosphate buffer, pH 7.4

Protein C	Time t_1	Relative viscosity η	Volume fraction of the protein ϕ	Specific volume of the protein $\phi \times 100$
grams per 100 cc.	seconds		per cent	
7.15	118.3	1.594	9.98	1.40
6.44	112.0	1.509	8.88	1.38
5.72	106.7	1.438	7.80	1.36
4.76	99.9	1.346	6.48	1.36
3.58	89.6	1.208	4.11	1.15
2.38	85.5	1.152	3.13	1.32
1.43	80.5	1.085	1.85	1.29
0.57	76.9	1.036	0.81	1.42
0.14	74.6	1.005	0.10	0.71
0.00	74.2*	1.000		

* This is the value for t_1

TABLE III

Specific volumes of serum and plasma proteins in late pregnancy as derived from viscosity measurements

Case number	Serum					Plasma				
	Protein C	Viscosity time t_1	Relative viscosity η	Volume fraction of protein ϕ	Specific volume of protein $\phi \times 100$	Protein C	Viscosity time t_1	Relative viscosity η	Volume fraction of protein ϕ	Specific volume of protein $\phi \times 100$
	grams per 100 cc.	seconds		per cent		grams per 100 cc.	seconds		per cent	
1	6.44	109.9	1.618	8.95	1.65	6.73	117.3	1.620	10.33	1.81
2	6.88	107.9	1.670	10.00	1.66	6.90	119.0	1.740	11.80	1.71
3	6.41	111.6	1.641	9.30	1.78	6.84	119.8	1.680	10.76	1.84
4	6.18	117.4	1.622	10.30	1.67	6.65	123.3	1.711	11.45	1.76
5	7.08	116.3	1.606	10.20	1.44	7.48	134.0	1.712	11.46	1.84
6	6.95	116.8	1.595	10.00	1.44	7.37	135.5	1.747	11.90	1.66
7	7.22	121.0	1.571	10.98	1.81	7.91	135.9	1.877	12.39	1.68
8	6.35	116.3	1.505	10.19	1.63	6.93	134.6	1.707	11.38	1.70
9	6.00	110.3	1.523	9.00	1.80	5.87	120.1	1.659	10.80	2.01
10	5.07	111.9	1.533	9.10	1.79	5.82	122.7	1.709	11.27	2.06
11	6.11	121.4	1.577	11.00	1.80	6.42	128.7	1.779	12.28	1.91
12	6.30	121.0	1.571	10.90	1.78	6.64	128.4	1.751	11.92	1.79
13	6.31	122.5	1.710	11.50	1.82	6.79	135.0	1.866	13.20	1.96
14	6.49	118.3	1.585	10.00	1.66	6.15	127.8	1.678	11.05	1.89
15	6.41	112.9	1.559	9.86	1.49	6.91	122.8	1.696	11.25	1.83
16	6.96	126.8	1.731	11.95	1.71	7.53	137.7	1.867	13.65	1.80
17	6.97	118.0	1.620	10.45	1.87	7.10	128.4	1.735	11.79	1.86
18	7.02	121.0	1.571	10.93	1.56	7.46	129.5	1.733	12.34	1.94
19	6.37	113.4	1.568	9.68	1.52	6.77	121.5	1.698	11.05	1.82
20	6.79	120.3	1.603	10.80	1.59	7.35	122.1	1.826	12.80	1.74
21	6.79	115.4	1.591	9.88	1.47	7.29	125.3	1.739	11.70	1.81
22	6.88	118.9	1.675	9.78	1.41	7.23	121.7	1.651	11.08	1.81
Average	6.37	117.0	1.610	10.25	1.61	6.82	125.8	1.738	11.79	1.74

* Potassium oxalate was used as the anticoagulant. Fibrin was determined directly and the value added to serum protein to obtain the total plasma protein values.

TABLE IV

Specific volumes of serum proteins in parturient women and their newborn children as derived from viscosity measurements

Case number	Maternal blood serum (parturient women)					Cord blood serum				
	Protein C	Viscosity time t_1	Relative viscosity η	Volume fraction of protein ϕ	Specific volume of protein $\phi \times 100$	Protein C	Viscosity time t_1	Relative viscosity η	Volume fraction of protein ϕ	Specific volume of protein $\phi \times 100$
	grams per 100 cc.	seconds		per cent		grams per 100 cc.	seconds		per cent	
1	4.30	118.4	1.650	10.70	1.70	4.76	97.5	1.318	6.48	1.26
2	5.96	120.2	1.600	10.82	1.23	5.24	107.8	1.459	8.58	1.81
3	6.78	124.1	1.714	11.50	1.70	6.10	108.0	1.492	8.60	1.41
4	6.83	125.2	1.745	11.25	1.99	6.27	106.8	1.469	8.10	1.81
5	6.03	125.2	1.709	11.81	1.81	6.29	105.1	1.483	8.05	1.28
6	7.01	119.0	1.644	10.65	1.51	6.00	107.5	1.483	8.53	1.40
7	7.29	121.5	1.718	11.50	1.88	6.87	97.5	1.317	6.45	1.14
8	6.64	126.5	1.743	11.85	1.81	6.80	106.9	1.477	8.40	1.46
9	6.44	120.0	1.657	10.80	1.68	6.23	97.0	1.340	6.35	1.19
10	7.07	124.5	1.770	11.85	1.63	5.84	101.1	1.394	7.22	1.30
11	6.43	119.3	1.649	10.65	1.86	6.09	104.4	1.412	7.90	1.80
12	6.96	122.6	1.693	11.23	1.61	6.38	101.0	1.365	7.20	1.20
13	6.91	122.7	1.625	11.25	1.62	6.26	100.4	1.351	7.08	1.83
14	6.79	123.6	1.735	11.78	1.72	6.98	107.4	1.453	8.90	1.43
15	6.70	124.7	1.779	12.80	1.67	6.70	107.0	1.478	8.40	1.47
16	6.83	121.7	1.584	9.85	1.74	6.12	100.4	1.387	7.10	1.39
17	6.00	117.7	1.681	11.08	1.85	6.68	106.6	1.500	7.70	1.86
18	6.54	122.4	1.601	11.20	1.71	4.74	96.2	1.279	6.20	1.31
19	6.81	117.8	1.677	10.40	1.85	4.79	106.3	1.373	7.03	1.47
20	6.44	120.2	1.709	12.49	1.81	6.42	106.3	1.408	8.25	1.53
21	6.10	115.8	1.567	10.00	1.82	6.16	100.9	1.360	7.19	1.39
22	6.11	115.9	1.561	10.70	1.78	4.87	99.3	1.304	6.88	1.37
23	6.47	118.8	1.638	10.67	1.60	5.85	101.8	1.402	7.32	1.80
24	6.88	120.7	1.667	10.96	1.67	6.41	106.6	1.472	8.32	1.33
Average	6.48	121.9	1.684	11.19	1.73	6.53	108.1	1.434	7.61	1.33

hence, if all other factors are equal, the specific volume of cord serum protein is lower than maternal serum protein.

DISCUSSION

No satisfactory reason can be given why the specific volumes of plasma and serum proteins differ in the same blood, and in the sera of parturient women and their newborn children. However, a number of interesting possibilities may be offered. Since plasma and serum proteins differ only by their fibrinogen content, it is quite probable that the general shape or configuration of the serum protein molecule has been changed due to the separation of fibrinogen. The distribution of the electric charges on these may also be altered. These factors must play a definite part in viscosity measurements which in turn might lead one to false conclusions regarding specific volume of plasma and serum proteins in solution.

Preliminary experiments were made to determine the effect of the anticoagulants, potassium oxalate and heparin, on the viscosity of serum and plasma. Approximately 3 to 4 mgm of potassium oxalate or 0.5 mgm of heparin were added to each cc of whole blood, and half of these amounts to serum.

To determine whether the specific volume of the protein remained constant under different dilutions as low as 0.14 per cent, serum was diluted with distilled water, with isotonic sodium chloride, and with *M*/15 phosphate buffer, pH 7.4.

The plasma and serum of 22 women in late pregnancy were analyzed for viscosity time and for protein concentration. The same analyses were made on the sera of 24 parturient women and of their newborn children (umbilical cord blood).

RESULTS

The viscosity of both serum and plasma is increased with the amounts of heparin employed but is not changed with potassium oxalate (Table I). Consequently, potassium oxalate was used routinely as the anticoagulant.

TABLE I
*The effect of potassium oxalate and heparin on the viscosity time of serum and plasma **

Number	Serum			Plasma	
	Without K oxalate	With K oxalate	With heparin	With heparin	With K oxalate
	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>
1	115.5	115.0		131.5	121.5
2	116.5	116.7		130.3	119.7
3	121.0	120.5		137.1	129.8
4	113.4	113.2	115.8	128.7	121.5
5	115.4	116.0	120.0	134.8	125.2

* Viscosity of serum and plasma are expressed in seconds as compared with 71.2 seconds for water at a temperature of 25.0° C \pm 0.02° C.

The results of diluting serum with distilled water, with isotonic chloride, and with *M*/15 phosphate buffer, pH 7.4, to give various protein concentrations are given respectively in Tables II—A, II—B, and II—C. The volume fraction of the protein in each experiment is decreased by the dilution, but its specific volume remains constant.

The protein content and relative viscosity of serum and plasma, together with the volume fraction and the specific volume of their respective

proteins in late pregnancy, are presented in Table III. In each case the specific volume of the serum protein is slightly less than that of the plasma protein, the average values being 1.62 and 1.74. The relative viscosity of serum is lower than that of plasma, but the decrease is disproportionate to the decreased serum protein, thus accounting for the lower specific volume of the serum protein.

The data on the serum from parturient women and their newborn children (cord blood), as given in Table IV, indicate that all of the constituents determined are definitely lower in cord blood serum than in maternal blood serum. The decreased relative viscosity of cord blood serum is not proportional to the decreased protein content,

TABLE II—A

The effect on the specific volume of the protein as determined by viscosity measurements of diluting serum with distilled water

Protein C	Time t_1	Relative viscosity η	Volume fraction of the protein ϕ	Specific volume of the protein $\frac{\phi}{C} \times 100$
<i>grams per 100 cc</i>	<i>seconds</i>		<i>per cent</i>	
7.20	116.4	1.634	10.50	1.46
6.72	113.3	1.590	9.93	1.48
5.76	105.8	1.486	8.50	1.48
4.80	98.9	1.389	7.10	1.48
3.36	89.3	1.254	5.00	1.49
2.88	86.6	1.216	4.30	1.49
2.40	85.0	1.194	3.90	1.62
1.68	80.3	1.128	2.70	1.61
0.72	75.4	1.058	1.30	1.81
0.00	71.2*	1.000		

* This is the value for t_2 .

TABLE II—B

The effect on the specific volume of the protein as determined by viscosity measurements of diluting serum with isotonic sodium chloride (0.90 per cent)

Protein C	Time t_1	Relative viscosity η	Volume fraction of the protein ϕ	Specific volume of the protein $\frac{\phi}{C} \times 100$
<i>grams per 100 cc</i>	<i>seconds</i>		<i>per cent</i>	
8.20	136.5	1.885	13.47	1.64
7.18	122.9	1.698	11.27	1.57
6.15	112.7	1.557	9.50	1.54
4.10	95.4	1.318	6.00	1.46
2.05	82.6	1.141	2.92	1.42
1.03	77.4	1.069	1.50	1.46
0.16	73.1	1.010	0.20	1.25
0.00	72.4*	1.000		

* This is the value for t_2 .

OBSERVATIONS ON THE ABSORPTION, DISTRIBUTION AND EXCRETION OF SULPHAPYRIDINE, DAGENAN OR M & B 693¹

By W. HURST BROWN, WILLIAM B. THORNTON AND J. STUART WILSON

(From the Department of Medicine, University of Toronto and the service of Dr. H. K. Detweiler, Physician in chief, Toronto Western Hospital, Toronto)

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Editorial comment has recently appeared in several of the leading medical journals urging the need of gathering data on the absorption, distribution and excretion of M & B 693 (Dagenan or sulphapyridine) in the human subject. It is evident that an accurate assessment of the potency and toxicity of this drug must await such information. The present communication is based on a study of 90 cases of pneumonia treated by sulphapyridine in the Medical Division of the Toronto Western Hospital between October, 1938 and May 1939.

From the beginning every case of pneumonia treated by chemotherapy was followed as closely as possible with respect to the dosage, fluid intake and urinary excretion, and daily estimations in the blood and urine of the total, the free and conjugated fractions of the drug. Whenever opportunity presented, specimens of blood, cerebrospinal fluid and pleural effusions were collected simultaneously for assay. Special precautions were also taken to obtain complete collections of urine over the days of actual treatment and for at least four days following cessation of dosage. The method used in all chemical estimations was the method of Marshall *et al.* (2).

Scheme of dosage

After the first 20 cases had been treated according to the plan advocated by Evans and Gainsford (1), it was decided to alter the schedule of dosage to permit of an earlier attainment of initially high concentrations of sulphapyridine in the blood. After this time the patients usually received 2 grams every four hours for 6 doses with decrements of $\frac{1}{2}$ gram every four hours or every six hours during each of the ensuing twenty-four hours (as illustrated in Figures 1, 2, 5) until treatment was discontinued. To the

same end the fluid intake was deliberately reduced to below 2500 cc. per day. In some instances an overemphasis on this point resulted in the fluid intake temporarily falling to levels too far below this figure, with results which receive comment later in this paper.

In Canada the distribution of 2-(p-aminobenzenesulphonamido) pyridine, or M. & B. 693 is effected by the firm of Poulenc Freres of Montreal² under the name of Dagenan. All of the drug used for oral dosage in the Toronto Western Hospital came from this source and was manufactured in France by the firm of Rhone-Poulenc. It has been accepted that, chemically Dagenan is identical with M. & B. 693 as distributed in England, and sulphapyridine as distributed in the United States. For this reason the term *sulphapyridine* has been used in this report to denote the drug except in the soluble form. For the sake of brevity in some of the figures the designation 693 alone is used and refers to the 2-(p-aminobenzenesulphonamido) pyridine stripped of all sodium and water of crystallization although reference is made to the conjugated or acetylated form as *conjugated 693*.

In March 1939 a supply of the sodium salt of sulphapyridine manufactured by the Calco Company was received through the courtesy of Dr. Perrin H. Long³ of Johns Hopkins Hospital. Later free samples of *Solu daganen* were received from the Canadian distributors. This preparation is a 33 per cent solution of the sodium salt of Dagenan contained in ampoules of 3 cc. content. Clinical trials of the drug in this form are still in progress.

OBSERVATIONS

Observations on oral dosage

All but 2 of the 90 cases treated at some time received sulphapyridine by mouth, some of these received supplemental intravenous, or intra-

¹ The authors wish to record their appreciation to Poulenc Freres for providing free supplies of Dagenan and *Solu daganen* for use in this study.

² The authors are pleased to acknowledge their indebtedness to Dr. Perrin H. Long and to Dr. E. K. Marshall of Johns Hopkins University for their help and cooperation. Dr. Marshall tested the drug against

³ This investigation was carried out with the aid of a grant from the Banting Research Foundation.

Fibrinogen, having a higher molecular weight than serum albumin or globulin, leads one to suspect it as being the main factor responsible for the increase in viscosity of plasma over serum. The concentration of other constituents, such as lipids and electrolytes, is presumably the same in the serum as in the plasma. The higher proportion of globulin in maternal blood (7), as compared with cord blood, probably explains the increase in the viscosity of the maternal serum containing a given weight of total protein, and hence, a higher specific volume. It has been shown by Nugent and Towle (8) that as the albumin-globulin ratio for a given concentration of protein solution increases, the relative viscosity is decreased, an observation which is in agreement with the present study.

If it is assumed that blood proteins are hydrated colloids, the data obtained by application of the empirical equation would indicate that the plasma proteins might be more hydrated than the serum proteins, and that the serum proteins in parturient women might be more hydrated than those of their newborn children. It has been reported by du Nouy (9) that the specific volume of rabbit serum protein in solution is 1.645 and in the dry state is 0.785.

Not all investigators agree as to the state of the water in blood plasma. Sunderman (10) concluded from freezing point experiments that, within 2 per cent, the limit of error of his measurements, the content of free water of serum is equal to the content of total water. From viscosity measurements and data on the protein concentration of blood serum, Fishberg (11) concluded that the actual volume of a protein in solution at any dilution is proportional to the dilution and that it is unnecessary to assume any hydration of the protein molecules. He further indicated that variations in urea and cholesterol concentrations have a profound influence upon the viscosity of blood serum.

In hydremia in children Jochims (12) found a parallel fall in plasma viscosity and protein. The specific viscosity remained constant, indicating that the excess water of the plasma in no way disturbed the colloidal nature of the proteins. He also stated (13) that the viscosity of the blood plasma in 21 normal children was not related directly to the protein concentration as demanded by Einstein's formula.

SUMMARY

1 Potassium oxalate does not change the viscosity of serum or plasma, but heparin produces an increase.

2 Dilution of serum with distilled water, isotonic saline, or with *M*/15 phosphate buffer solution to a protein concentration as low as 1 per cent does not alter the specific volume of the protein.

3 The specific volume of plasma protein is slightly higher than that of serum protein from the same blood sample.

4 The specific volume of serum protein is higher in parturient women than in their newborn children (cord blood).

5 Factors involved in the variation of the specific volume of proteins are discussed.

BIBLIOGRAPHY

- 1 Kunitz, M., Empirical formula for relation between viscosity of solution and volume of solute. *J General Physiol*, 1926, 9, 715.
- 2 Einstein, A., Quoted by Kunitz (1). *Ann Physik*, 1906, 19, 289, 1911, 34, 591.
- 3 Hatschek, E., Die Viscosität der Dispersoide. Quoted by Kunitz (1). *Ztschr f Chem u. Indust d Kolloide*, 1910, 7, 301.
- 4 Hawk, P. B., and Bergeim, O., *Practical Physiological Chemistry*, P. Blakiston's Son and Co., Philadelphia, 1931, 10th ed., p. 449.
- 5 Folm, O., and Wu, H., A system of blood analysis. *J Biol Chem*, 1919, 38, 81.
- 6 Chandler, J., Determination of fibrin in blood plasma. *J Lab and Clin. Med*, 1927, 12, 1092.
- 7 Achard, C., Bariety, M., and Codouris, A., L'équilibre protéique du sérum comparé dans le sang de mère et le sang du cordon ombilical. *Compt rend Soc. de biol*, 1929, 102, 984.
- 8 Nugent, R. L., and Towle, L. W., Albumin-globulin ratios in synthetic solutions from specific gravity and relative viscosity measurements. *Proc. Soc. Exp Biol and Med.*, 1935, 33, 374.
- 9 du Nouy, P. L., Viscosity of blood serum as function of temperature. *J General Physiol*, 1929, 12, 363.
- 10 Sunderman, F. W., State of water in blood serum. *Am J M Sc.*, 1931, 181, 154.
- 11 Fishberg, E. H., Significance of changes of viscosity in pathological sera. *J Biol Chem.*, 1929-30, 85, 465.
- 12 Jochims, J., Sur Frage der Wasserbindungsverhältnisse des kindlichen Blutplasmas. *Klin Wchnschr*, 1930, 9, 2115.
- 13 Jochims, J., Viscosimetrische untersuchungen über die Wasserbindung der Plasmakolloide, die Wasserbindung der Eiweisskörper im normalen Blutplasmas. *Arch. f a ges Physiol.*, 1932, 230, 255.

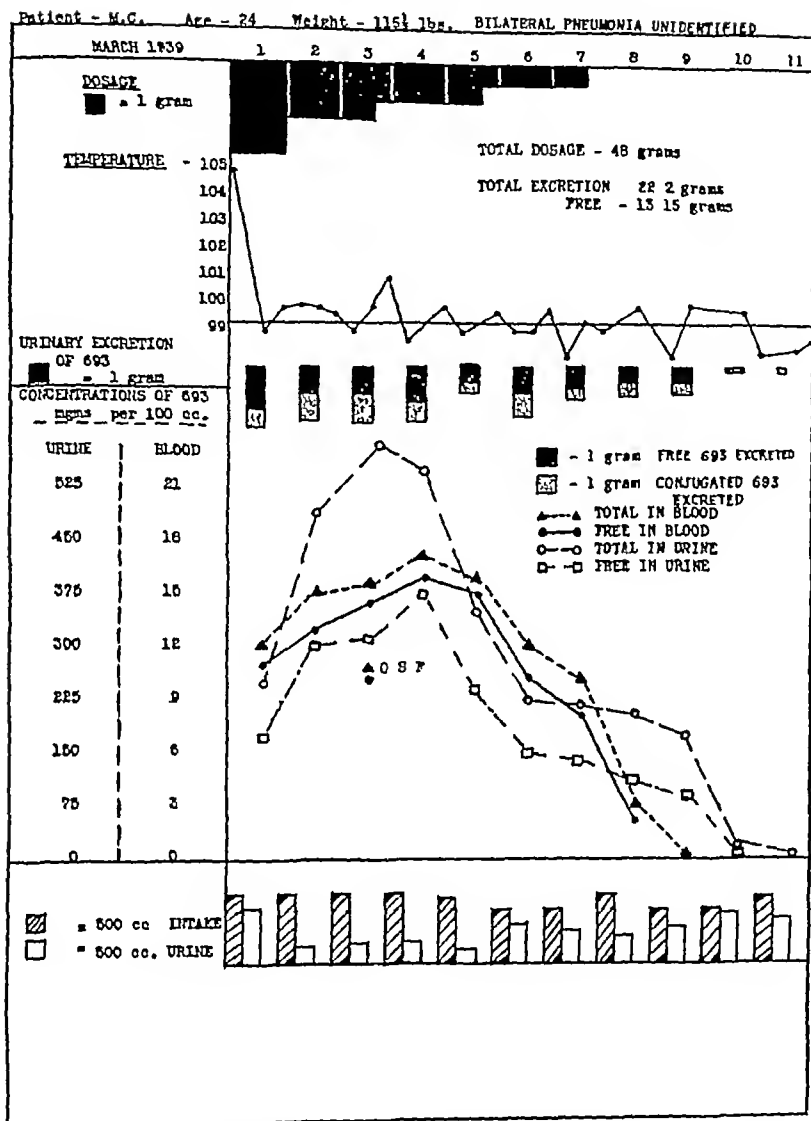


FIG. 1 A TYPICAL CASE OF HEAVY DOSAGE OF DAGENAM

Note the parallelism between the concentrations of the free and total drug in the blood, the levels in the cerebrospinal fluid, the time required for complete excretion of the drug. The excretion of the free form in this case was unusually rapid especially in the first 24 hours.

muscular sodium sulphapyridine or Solu-dagenan. General observations on the results of oral dosage will first be presented and the tentative conclusions reached will be subjected to criticism in a later part of the report dealing with cases treated by injection.

Observations on absorption

It was early and frequently observed that, independent of the factors of weight, fluid intake, vomiting, sweating, bowel activity, urinary excretion and gastric acidity, there was a wide discrepancy in the levels obtained in the blood on a standard scheme of dosage. In the entire series the concentration of non-conjugated sulphapyridine hereafter called *free* in the blood varied from 2.2 to 12.8 mgm per 100 cc at the end of twenty-four hours of therapy. After due allowance was made for all the factors named, there still remained gross differences. These variations must, therefore, be chiefly attributed to differences in the rate and degree of absorption of sulphapyridine from the gastro-intestinal tract. Some have stated that the rate of absorption is increased if the tablets are administered as a powder. Confirmation of this is not possible in the present series. The stomach of one patient who died ten minutes after receiving four 0.5 gram tablets of *Dagenan* by mouth was examined thirty minutes postmortem and found to contain amorphous material giving a positive test for sulphapyridine. No sign of the tablets, as such, could be found either in the esophagus, stomach or duodenum.

Observations on distribution

(a) The drug is apparently absorbed in the free or nonacetylated form. Conjugation usually takes place gradually with a resulting depletion of the free form. For this reason the highest relative values for the free form in the blood are commonly found in the first day of treatment.

(b) Although in most cases a parallelism exists between the levels in the blood of the free and conjugated forms from day to day (Figures 1, 2), in about one-fifth of the cases this is not true. In these instances, there appeared to be an unusually rapid conjugation of the drug and it was not infrequently found that an estimation of the free form alone gave no reliable indication of the total concentration of the drug in the blood

(Figure 3). This was particularly noticed in cases which developed renal insufficiency. In these the concentration of the total sulphapyridine in the blood often rose concurrently and abruptly to very high levels (Figures 5, 6). Since the power to conjugate the drug was apparently not impaired, concentration levels of the total and free forms rapidly diverged. In some of the cases, indeed, it seemed that the attainment of high blood levels accelerated the rate of conjugation (Figures 5, 6).

In view of Marshall's statement (3) that acetylated sulphapyridine is more toxic than the free form, these findings have a manifest importance. Some of the serious toxic effects were observed to occur in those cases which, at some time or other, showed inordinately high values in the blood of the conjugated fraction.

(c) In only one of 16 cases in which the concentration of the cerebrospinal fluid was estimated at the same time as the blood, was the level in the cerebrospinal fluid as high as that in the blood. In the majority it was about 65 per cent of the blood concentration (Figures 1, 2). In all instances, a disproportionately high percentage of the free form of the drug was present in the cerebrospinal fluid as compared with blood values simultaneously obtained. In 8 of 16 cases there was practically no conjugated drug in the spinal fluid.

(d) A large number of the estimations on the pleural fluid were rendered suspect by the use of novocaine as an anaesthetic. In those samples obtained without using an anaesthetic the values were never less than the concentration in the blood.

(e) The drug was recovered from the vomitus, sputum, and feces of patients under therapy. In cases on oral dosage the amounts recoverable in the feces were very large indeed.

(f) Chemical assays of the organs of 4 cases showed that sulphapyridine is unequally distributed among them in terms of concentration per 100 grams of tissue. It is high in the kidney (where contamination by urine is an important factor) and next highest in the liver. It is variable in the spleen and low in the brain, bile, and body fat. In these tissues the percentage of drug in the conjugated form was found to be lower than in the heart's blood.

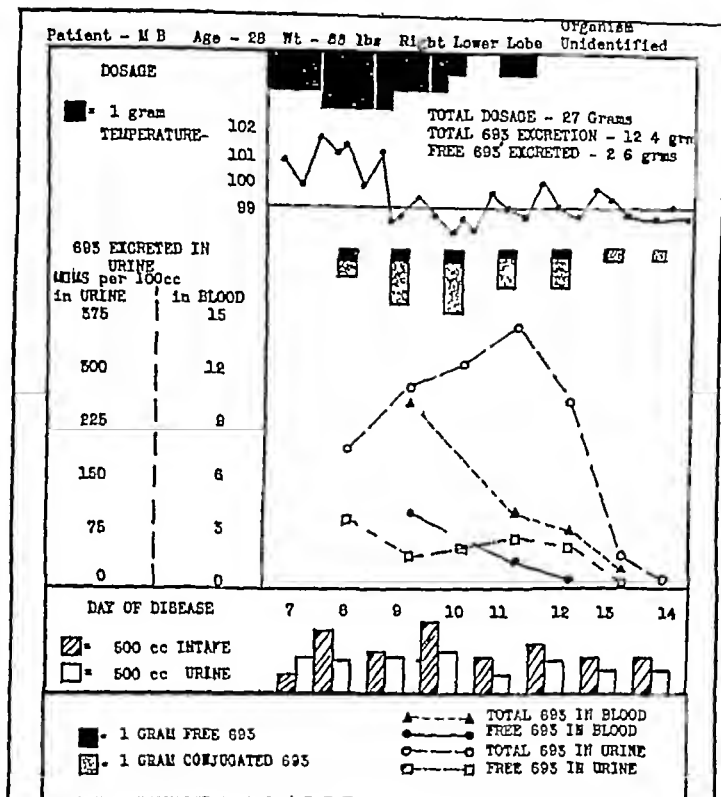


FIG. 3 AN EXAMPLE OF RAPID CONJUGATION WITH RAPID EXCRETION OF THE ACETYLATED FORM IN THE URINE

concentration of total drug in the urine to the concentration of total drug in the blood reach 40. In the great majority it was nearer 20 to 25. This is in contrast to experience with sulphanilamide where the ratio may often reach 40. This finding may have a bearing on the relatively slow excretion of sulphapyridine from the body. In the surviving cases which were treated by oral administration alone, the last measurable quantity of sulphapyridine disappeared from the blood in sixty hours and from the urine, excepting very faint traces, in ninety six hours after the final dose had been given (Figures 1, 2, 4, 5, 6).

(b) In view of the above findings it was anticipated that a very considerable proportion of the total amount of drug ingested would be recovered in the urine (6). Even when the blood concentrations were high indicating a high degree of absorption, the amounts recovered in the urine were remarkably low (Figures 1, 2, 3, 4, 6). In only 3 cases in which absorption was apparently very rapid, was as much as 50 per cent of the total sulphapyridine given by mouth recoverable in the urine and in most instances less than 33 per cent was recoverable.

(c) It was generally found after the first

Observations on excretion

(a) There can be no doubt that the urine constitutes the main channel of excretion of sulphapyridine from the body. This is particularly well illustrated in cases in which renal function is defective (Figures 5, 6, 7). Provided that urinary excretion was not impaired, the administration of large quantities of fluid by mouth, or

especially by vein, decreased the blood concentrations rapidly (Figures 2, 5, 6)

The kidney has the power of concentrating the drug to a very remarkable extent. Five cases were seen in which the concentration of total sulphapyridine in the urine rose to over 500 and in 3 cases to over 600 mgm per 100 cc. of urine. In only one of these, however, did the ratio of

Patient - W L Age - 36 Weight - 157½ lbs. PNEUMOCOCCUS TYPE IV. RIGHT LOWER LOBE.

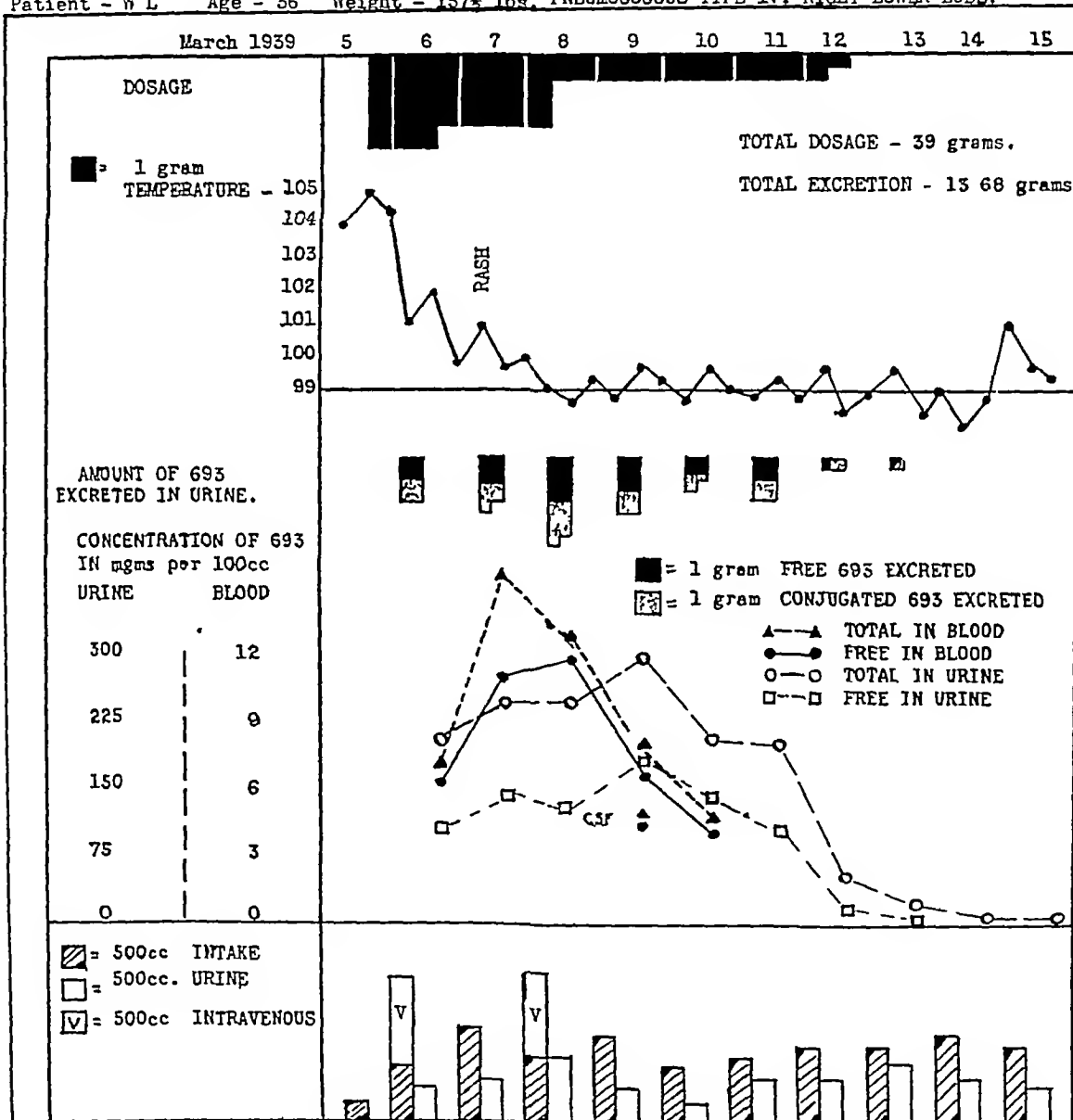


FIG 2. THE EFFECT OF FLUID INTAKE ON THE BLOOD LEVELS AND ON URINARY EXCRETION OF THE DRUG

Total excretion is complete in 72 hours. Note CSF Levels. Note rate of excretion of free and conjugated forms in the urine compared with the blood levels.

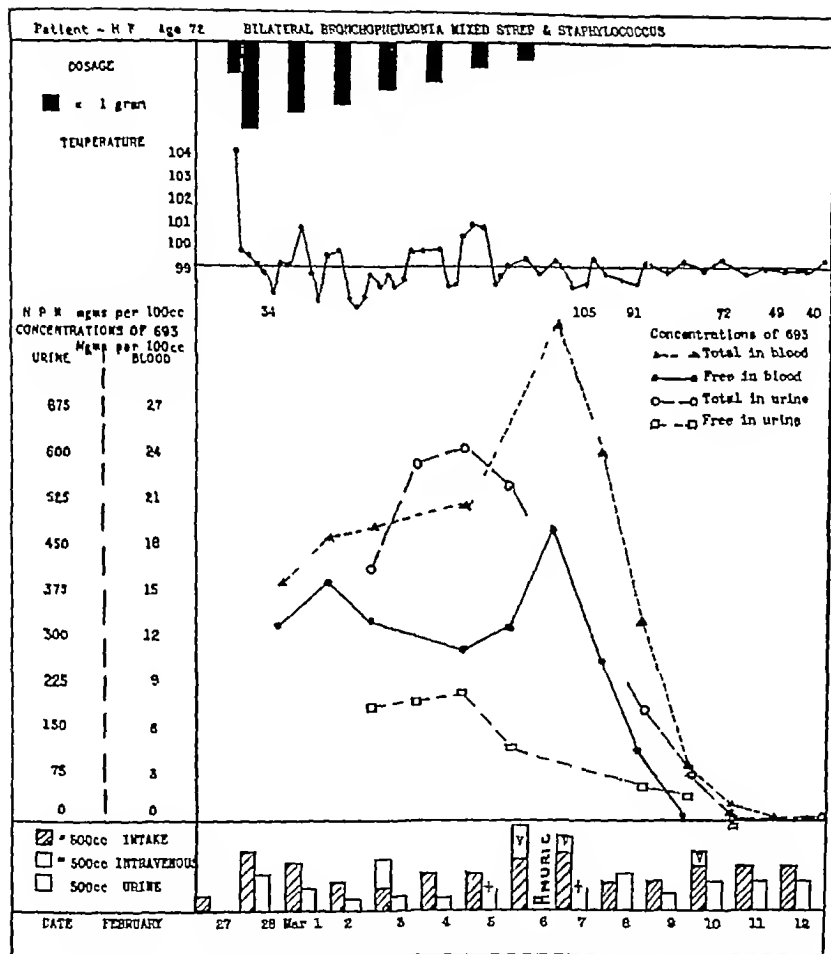


FIG. 5 THE EFFECT OF OLIGURIA AND ANURIA (ACUTE RENAL INSUFFICIENCY) ON THE N.P.N. AND THE CONCENTRATIONS OF THE DRUG IN THE BLOOD

There seems to be an acceleration of the rate of acetylation. Note the rapidity of excretion following restoration of renal function.

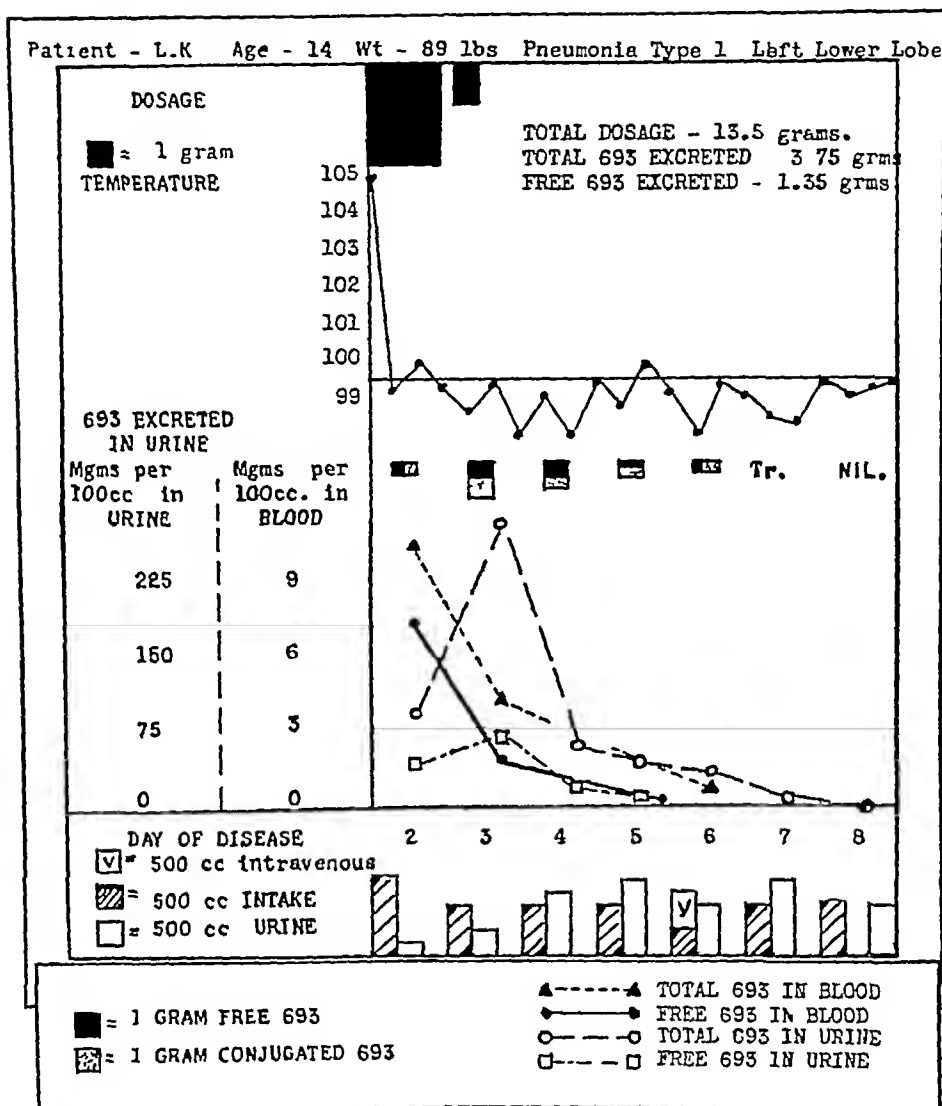


FIG 4 POOR ABSORPTION OF ORAL DOSES AND COMPLETE EXCRETION IN 96 HOURS

twenty-four hours that the free form was not as rapidly excreted as the conjugated form (Figures 1, 2, 3, 4, 6). The occurrence of oliguria (less than 300 cc of urine excreted in twenty-four hours) in 6 cases and of anuria in 3 cases during treatment was associated with abnormally high blood NPN values, an increase in the total concentration of sulphapyridine in the blood, and a marked and very rapid rise in the blood concentration of the conjugated fraction (Figures 5, 6). Moreover, although there were exceptions, it was the rule that the concentration of the free form in the urine was disproportionately low as compared to the concentration of the total sulpha-

pyridine in the urine, having regard for the existing levels of both in the blood at the time.

Intravenous and intramuscular injections

There are obvious disadvantages attending an attempt to draw conclusions pertaining to the title of this report from clinical cases receiving prolonged dosage by mouth of a highly insoluble drug. Comparisons of the concentrations attained in blood and urine with the dosage are open to too many undeterminable influences. For this reason, it is of interest to record in more detail the observations in 4 of the cases in which injections of the soluble form of the drug were

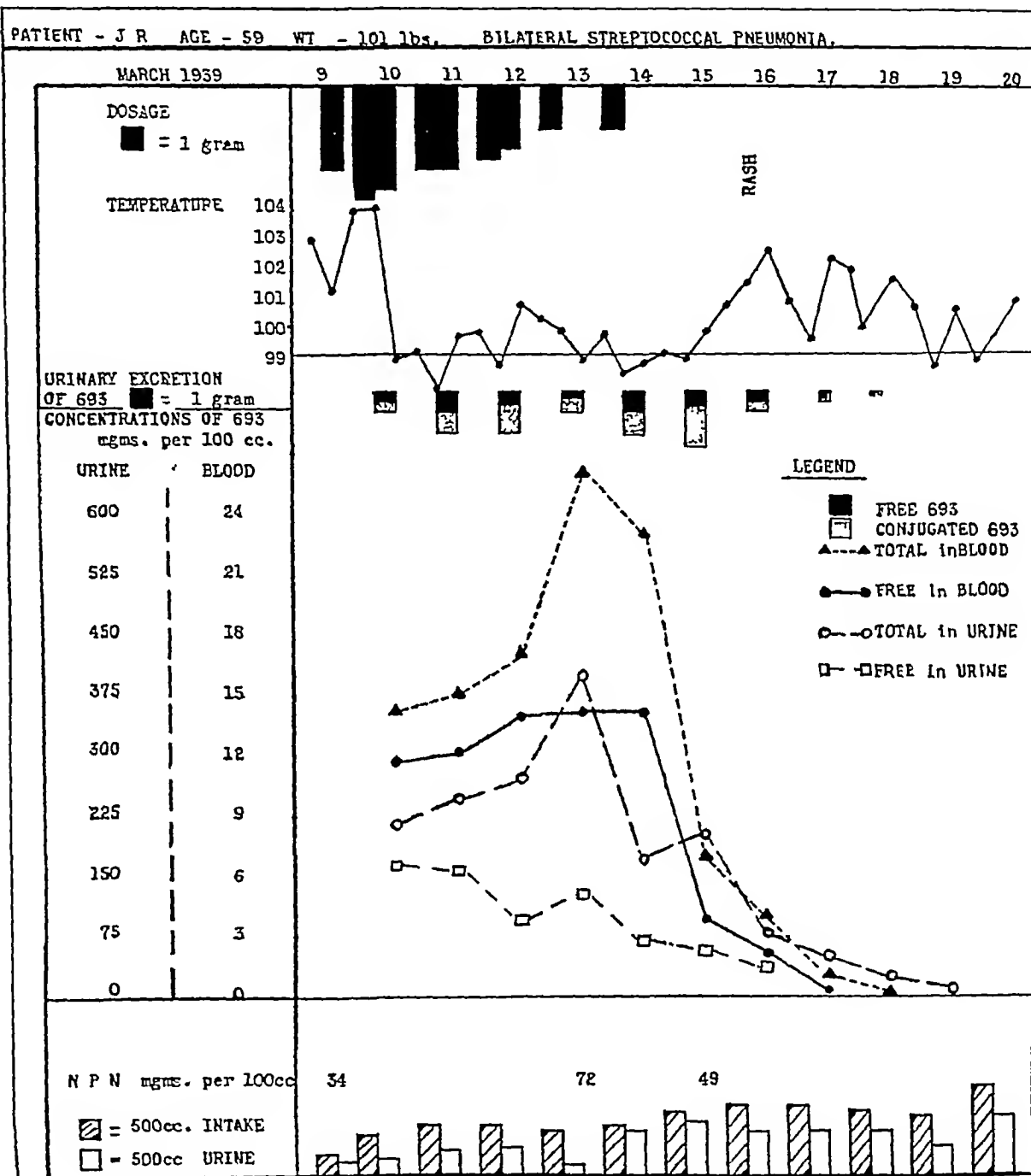


FIG 6 THE EFFECT OF ACUTE RENAL INSUFFICIENCY (COMPARE FIGURE 5), POSSIBLY DUE TO AN INADEQUATE FLUID INTAKE ON THE N P N AND CONCENTRATIONS OF THE DRUG IN THE BLOOD

An abrupt rise in the rate of acetylation (see blood levels) coincides with oliguria. Extremely rapid excretion follows the discontinuance of chemotherapy and restoration of renal function without a great increase in the fluid intake. Note excretion of the conjugated fraction.

used. From a study of these it was expected that more definite pronouncements regarding the tentative conclusions already noted might be justified.

PROTOCOLS

Case 1

In the first case, a boy of 16 weighing 134 pounds, 2 intravenous injections of 3 grams each of 5 per cent sodium sulphapyridine were made at nine-hour intervals. The blood concentrations, attained half an hour after the first injection was completed (fifteen to eighteen minutes were taken to inject the fluid), were 69 mgm. per 100 cc. total drug, and 64 mgm. per 100 cc. free. Nine hours later, and immediately before the next injection, the values were 54 total and 48 free. Eight and sixteen hours after this second injection the levels were respectively 70 total and 66 free, and 58 total and 45 free. In the first twenty-four hours the fluid intake was 3,000 cc. and the urinary output, which contained 147 grams of sulphapyridine, was 1,100 cc. Sulphapyridine continued to be excreted in measurable concentrations in the urine until the fourth day, and in traces until the sixth day. Measurable concentrations of the drug in the blood were present until the second day after the last injection, and in traces for the next two days. Although 6 grams in all of the sodium salt were injected, the actual amount of sulphapyridine was 5.16 grams. Of this quantity 3 grams or 60 per cent were excreted as conjugated and free sulphapyridine in the urine, only 185 grams or 36 per cent being in the free form.

This patient made a rapid and uninterrupted recovery from pneumococcal pneumonia Type II without further chemotherapy. The Francis test became positive forty-eight hours after admission (the 5th day of his disease).

Case 2

The second case requires a more detailed exposition. A young man (Figure 7), 19 years old, weighing 148 pounds, was admitted in a moribund condition on the night of April 16, 1939. He was very pale, restless and continually grimacing. Pulse—110, respiration—30, temperature—105, and blood pressure 120/60. The physical signs were those of frank consolidation of the left lower lobe, but his condition was disproportionately grave considering the apparent extent of lung involvement. Oral therapy by sulphapyridine was impossible and it was thought wise to administer intensive treatment with sodium sulphapyridine intravenously at once. This was given as a 5 per cent solution in doses of 3.8 grams in 70 cc. of distilled water. Fifteen to eighteen minutes were taken for each injection. Five minutes after each injection was completed, a sample of blood was taken from the opposite arm, except on one occasion (after the second injection), when twenty minutes elapsed before the sample was taken. After the third injection the results of cultures were reported. The blood cultures were consistently negative. *Pneumococcus* Type XXIII and *staphylococcus aureus*

were present in the sputum. On mouse passing both were again recovered but the heart's blood of the mouse yielded only *staphylococcus aureus* in pure growth. This was considered to be a significant indication of the invasive properties of the *staphylococcus*. The pneumonic process seemed to be extending although the temperature had fallen from 105 to 103 and the patient seemed slightly better. It was now considered likely that the *staphylococcus* was the responsible organism and in view of reports (4) in the literature attesting cures of *staphylococcal aureus septicaemiae* by M & B 693, it was decided to give another injection after an eight-hour interval in the hope that by maintaining high blood levels a cure might be obtained.

No untoward reaction to intravenous injections had previously been experienced but, during the fourth injection phase, the great restlessness of the patient abruptly gave way to an ominous quietude, almost as if an anesthetic were being administered. There was a period of collapse and the blood pressure fell from 115/70 to 95/50. These events were clearly the results of the injection and largely disappeared in forty-five minutes when the blood levels were at a demonstrably lower level. A comparison of Figures 7 and 8 illustrates how much the blood levels anticipated in view of previous cases were exceeded by the third and fourth injections. This unexpected and undesired accumulation of the drug must again be attributed to renal failure—in part, no doubt, abetted by the low filtration pressure. Complete anuria was present during the last ten hours of life and, in the light of past experience, it is reasonable to attribute part of the renal failure to the direct action of the drug. It is noteworthy and, under the circumstances, to be expected that the administration of almost 2500 cc. intravenously of 5 per cent glucose in saline during the twenty hours following the fourth injection had, in this instance, no effect in combatting the enormous accumulation of the drug. It is proof positive of the importance of free renal excretion in relation to intravenous or oral therapy with chemicals of this type.⁴

Another finding of importance is illustrated by Figures 7 and 8—namely, that immediately after any intravenous injection, extraordinarily high concentrations of the drug are present in the blood. After a few minutes these fall rapidly and in thirty to sixty minutes distribution has been completed. It is not easy to understand, however, how the body is able to conjugate the free form as rapidly as is demonstrated in these cases. In the case under consideration, excretion of the free form is not as rapid as the excretion of the conjugated form. This is shown by the relatively more rapid fall of the total levels and by the relative amounts of the two forms in the urine first secreted. After the fourth injection, urinary excretion was practically at a standstill and there was no appreciable fall in the total concentrations in the blood. The concentrations of the free form did, however, gradually fall owing to the continuance of acetylation.

⁴ The pH of solutions of sodium sulphapyridine or Solu-dagenan as used in these cases was about 11.

amounts injected than would be expected from the results of chemical assay of the various organs, although these after all constitute only a very small percentage of the total body weight

Case 3

A third case, successfully treated by intravenous sodium sulphapyridine, is illustrated in Figure 8. This young man was aged 21 and weighed 138 pounds. He was admitted on April 26 1939 with Type III pneumococcal pneumonia as shown by blood and sputum cultures and by mouse inoculation. He received intravenously two injections of 3.8 grams, followed by two injections of 2 grams of sodium sulphapyridine at the intervals noted in Figure 8. He responded well to this therapy but later developed an empyema for which he was treated with Dagenan by mouth for three days until it was established that only staphylococcus aureus was present in the pleural pus and resort was had to surgical treatment.

In this case no impairment of renal function occurred although 116 grams of the sodium sulphapyridine were injected over a period of forty-eight hours. There was no sign of accumulation of the drug. The administration of 5170 cc. of fluid (3000 cc. intravenously) in the twenty-six hours following the last injection reduced the blood level from 7.5 mgm. per 100 cc. of the total drug per 100 cc. blood to a trace. Complete figures are available for urinary excretion of both the free and total moieties for the first seventy-two hours. These are represented with mathematical exactitude in Figure 8. It will be seen that expressed as pure or free sulphapyridine, the patient received a net total dosage of 10 grams after making allowance (1.62 grams) for sodium and water of crystallization. Of this total amount 51 grams had been excreted in the urine at the end of seventy-two hours and of this only 2 grams were in the free form.

It is of particular interest to compare the blood levels attained in this patient on intravenous therapy with those later attained when sulphapyridine was given by mouth. The daily fluid intake was purposely maintained at 2000 cc. during this period. In Table II the results are noted, each blood sample being taken four hours after the preceding dose.

Twelve hours after dosage was discontinued only a trace was present in the blood.

It is evident that the plan of dosage by mouth maintained a more constant level in the blood. In all, 27 grams were given by mouth over a period of seventy-two

hours, as against 10 grams in the earlier period of seventy-two hours of intravenous therapy without achieving significantly higher levels. It must be concluded therefore, that more than half of the sulphapyridine given by mouth was not absorbed.

Case 4

The following case admitted with pneumococcal pneumonia on May 7 1939 the first day of his disease, deserves mention since it is one of the 4 cases on injection treatment which were studied closely. The protocol of this man is shown in Table III. It will be noted that he was first treated with intramuscular Solu-dagenan alone and two days later sulphapyridine by mouth. The intramuscular injections consisted of 33 per cent Solu-dagenan put deeply into the gluteal muscles. At first he reacted well to intramuscular therapy but on the second day (May 9) his infection escaped from chemotherapeutic control although his levels were still high. Despite a falling leukocyte count, clinical signs of extension of the pneumonia ruled out chemical toxicity as the cause of the increased fever. Sulphapyridine therapy by mouth in full doses was therefore begun with an ensuing improvement in his general condition and a fall in temperature. The white count now fell to what experience has shown to be ominously low levels (total granulocyte count—2,200 per cm.). The drug was then discontinued and the administration of very large quantities of fluid by mouth and 10 per cent glucose saline intravenously was begun. This resulted in a very rapid removal of the drug from the body. The total leukocyte count began at once to rise but even ten days later the polymorphonuclear cells were only 60 per cent of the total leukocytes. The patient developed a hepatitis as noticed in the protocol and finally proceeded to a complete cure, being discharged from hospital on May 28 1939.

The following points are of importance

1 The blood concentrations attained after intramuscular injections are naturally not characterized by the sharp peaks already seen in cases treated intravenously. There is on the other hand a gradual but fairly rapid rise in the blood levels which reach their maximum in about three to three and a half hours after which, with normal fluid intake, they begin slowly to fall. The greatest increments after each injection occur when the initial blood levels are low—thus 2 grams at first raised the total concentration in the blood from zero to 4.2 mgm. per 100 cc. and the free form to 4 mgm. per 100 cc. in three hours while the fourth injection of 1 gram raised the concentrations from 8.2 total and 6.7 free to 9.8 total and 7.9 free in three hours.

2 It seems that the higher the concentration of the drug in the blood the more rapid the con-

TABLE II

Blood concentrations (free) reached in patient IV S
(Figure 8) on oral administration of Dagenan

Dosage grams	Blood concentration of free drug 100 cc.
1 dose of 2 grams	3.8
After 2 grams q 4 h for 4 doses	7.1
After 2 grams q 4 h for 6 doses	5.7
After 1½ grams q 4 h for 6 doses	5
After 1 gram q 4 h for 6 doses	4.2

An autopsy held one hour after death established the following anatomical diagnosis: bilateral lobar pneumonia, multiple lung abscesses, bilateral pleurisy with effusion, pleural adhesions, cerebral edema, fatty liver, septic spleen, toxic renal damage.

The abscesses were found in all five lobes of the lungs. From these staphylococcus aureus was cultured in pure growth. No pneumococci were present in any cultures from the lungs. The cultures of the blood from the heart yielded staphylococcus aureus only.

The kidneys were described as being slightly swollen with widening of the cortex and bulging of the cut surface. No hemorrhages were present. Microscopic studies showed a moderate degree of disintegration of the cells of the first convoluted tubules and deposits of pigmented chemical crystals lying in the tubules. No casts were seen and only a small amount of debris was contained in the lumina of the tubules. There was no evidence of actual obstruction of the tubules. The glomeruli were apparently normal.

The liver had obviously undergone much fatty change and on microscopy this was easily seen in fine globules uniformly distributed throughout the liver cells in all areas. This has invariably been noted in all our cases dying while on sulphapyridine therapy. It is, however, impossible, in our view, to attribute these changes to the employment of sulphapyridine in treatment. The most striking case of liver damage encountered received 10 grams of the drug by mouth in twenty-two hours but the maximum concentration attained in the blood was only 4.9 total and 2.2 free mgm per 100 cc. The liver was so fatty that it floated easily in water but the damage should, in this instance, be attributed to the toxicity resulting from the disease rather than chemotherapy.⁵

The organs and various fluids and tissues were subjected to chemical analysis for their content of sulphapyridine with results as noted in Figure 7. It will be noted that the heart's blood, pleural fluid, and pericardial fluid yielded high assays for the drug. The lung (consolidated) also had very high concentrations as had the kidney. The other organs and the bile yielded smaller concentrations. As has previously been pointed out, the CSF had a relatively high content of the free form but much less conjugated drug than the blood. The brain in this instance contained none of the conjugated drug. This result has been confirmed in 3 other cases in which the amount of the conjugated form never exceeded 5 per cent of the total. No explanation can at present be offered for this remarkable finding.

The bowels had not been emptied since chemotherapy was begun. It is therefore of interest to compare the concentration of the drug found in the feces from the rectosigmoid junction with that in the feces from the cecum and in the bile. The findings indicate that an appreciable amount of the drug is excreted into the bowel independently of the bile, but that the concentrations are not high in the bowel content.

⁵ The authors wish to thank Dr. George Shanks for his reports on the autopsy material.

The lowest concentrations were obtained in body fat taken from the abdominal wall and, here again, the free form greatly predominated. The following were the total quantities contained in the various organs:

TABLE I
Sulphapyridine

Organ or material	Weight	Free mgm		Conjugated mgm	
		In 100 grams	Whole organ	In 100 grams	Whole organ
	grams				
*Lung (2)	1850	18.0	351	3.0	58.5
Kidneys (2)	335	14.0	47	4.0	11.0
*Liver	2050	10.0	201	1.0	20.5
Spleen	300	6.0	18	1.0	21.0
*Brain	1650	6.0	106		
*Body fat		4.5		0.8	
*Feces					
Caecum		6.0		1.0	
Recto sigmoid		5.0		1.0	

* Chemical assay was carried out on only a portion of the total organ or material in these instances and the possibility of unequal distribution of the drug is not precluded.

It seemed possible, before 4 fatal cases were investigated in this way (with results which throw much doubt on the validity of such calculations), to estimate the total amount of drug in the body from the concentrations in the blood and the body weight. Such calculations depend upon the assumption that the sulphapyridine is distributed in almost equal concentrations to all tissue fluids and it is apparent that this may not be the case. Using Peters' researches (5) as a basis, and by the arbitrary assumption that 70 per cent of the body weight is distributable fluid of a specific gravity (for pooled fluid) of about 1.020, the amount of sulphapyridine in the body one hour after the first injection is by calculation 3.1 to 3.2 grams and two hours after the fourth injection about 11.8 grams.

Each of the four injections contained 3.8 grams of sodium sulphapyridine. Almost exactly 14 per cent of sodium sulphapyridine is made up of sodium and water of crystallization, therefore the amount of sulphapyridine contained in each injection is actually not 3.8 grams but about 3.3 grams. At the end of the first hour, therefore, a little less than 3.3 grams should be present in the body and two hours after the last injection, 13.2 grams minus the amount lost in vomitus, saliva, sweat and urine should be present. The calculated values bear a closer relationship to the actual

TABLE III—Continued

Dose	Date	Hour	Concentration of Dapsone in blood		Concentration of Dapsone in urine		Urine volume	Dapsone excreted in urine		Fluid intake	Rectal temperature	White blood cells	Red blood cells	Hemoglobin	Remarks
			Total	Free	Total	Free		Total	Free						
1½ grams Dapsone per Os.			mgm. per 100 cc.		mgm. per 100 cc.		c.c.s. centigrade	grams		cc.			millions per c.mm.	per cent	
		4:00 p.m.	15.3	15.5							99.0				Neutrophils only 45 per cent Dapsone discontinued
		8:00 p.m.	17.6	14.7						8,000	100.0	4,900			Flakes by mouth and intravenously
	May 11	12:01 a.m.			122	78	1,800	1.97	1.25		99.8				
		9:00 a.m.			170	112	2,500	4.15	2.80		98.8	7,500	8.10		Neutrophils 86 per cent
		11:30 a.m.	12.3	11.7	221	112	350	0.73	0.59		99.2				
		4:00 p.m.	10.4	9.4	139	83	650	0.90	0.57		99.2				
		11:00 p.m.	6.9	5.9	82	57	1,500	1.45	1.03	4,800	100.3				
	May 12	9:00 a.m.	4.1	3.8	73	45	1,900	1.29	0.87		101.5	5,900	4.77	84	Signs of pneumonia extending
	May 12	8:00 a.m.	1.6	1.5	42	28	2,450	1.03	0.69	8,050	103.4	100.3			Van den Bergh 1.84 units
Total		7:00 p.m.	Trace	Trace v. a.	14	9	1,550	0.26	0.17		102.3				Icterus obvious
8 grams Solu-dapsone, intravenously plus 15 grams Dapsone by mouth	May 14	7:00 a.m.	Slight trace	Slight trace	35	19	400	0.14	0.08	3,200	100.0				Rash on abdomen
		7:00 p.m.	v. a. trace	ND	7	5	2,000	0.14	0.100	3,500	102.6				Van den Bergh 13 units
	May 15	7:00 a.m.	Slight trace	Trace	10	6	400	0.64	0.02		100.0				Van den Bergh 15 units
		7:00 p.m.	ND		12	7	1,450	0.17	0.10	2,500	102.4	11,400	4.30	82	Liver 2½ inches below right costal margin
	May 16	7:00 p.m.		Trace	EL. trace		480			2,800	99.8				
	May 17	7:00 p.m.		EL. tr. v. a. tr.			2,000			2,200	99.0	3,500	4.13	80	Van den Bergh 22 units
Total dosage 22 grams	Totals						22.3 Liters	19.00	12.13	35 Liters					Liver receding. Thereafter Van den Bergh reading and normal on May 23. Complete recovery
Hereafter traces were detected in the urine until May 28th, or eighteen days after the last dose of Dapsone had been given.															

May 15 until May 28) This patient, therefore, excreted about 85 per cent of the drug originally administered

No other case in the entire series showed such a prolonged excretion and the assumption seems tenable that this was due to delay in freeing the body of the Solu-dapsone injected intramuscularly. If this should prove to be the case it is a point worth consideration in view of the proven toxicity of drugs of this type.

DISCUSSION

In addition to the points which have been made, other impressions already recorded with respect to cases treated with sulphapyridine by mouth re-

ceived support from the studies in these 4 cases treated by injections. At the risk of repetition certain points merit emphasis.

It is clearly evident that there must be wide variations in the ability of the human subject to absorb the drug from the gastro intestinal tract. From two to three, four and even five times as much sulphapyridine must often be given by mouth to attain the concentrations in the blood observed after injection of the soluble sodium salt. By inference, and it is proven by analysis much of the drug given by mouth must pass unabsorbed through the gastro intestinal tract. This is an unsatisfactory aspect of this form of chemotherapy. It makes necessary frequent

jugation This has already been remarked upon in the cases receiving sulphapyridine by mouth. It is, however, noteworthy that the period during which liver damage became evident (May 12 and 13) coincided with a very low concentration in the blood of the conjugated fraction. As the liver has been stated to be the chief, if not the only, organ concerned in acetylation (7), it is possible that the marked diminution of the conjugated fraction in the blood after May 12 is thus explained. It must be borne in mind, however, that there was also a very rapid excretion of the drug in the urine during this period.

3 The substitution of sulphapyridine by mouth for intramuscular injections on May 9 resulted in a rapid absorption of the drug and the attainment of very high levels in the blood (18.3 mgm total and 15.5 mgm free per 100 cc.) The unusually large amounts absorbed were more completely excreted in the urine than in any other case in the series. The total dosage administered by both methods was something less than 23 grams (allowing for sodium in the molecule of Solu-dagenan) and the amount excreted in the urine was somewhat more than 19 grams (Traces continued to appear in the urine from

TABLE III

Patient, K. H. Age 32 Weight 124 pounds Type III pneumococcal pneumonia, right lower lobe
Blood cultures repeatedly negative

Dosage	Date	Hour	Concentration of Dagenan in blood		Concentration of Dagenan in urine		Urine volume	Dagenan excreted in urine		Fluid intake	Rectal temperature	White blood cells	Red blood cells	Hemoglobin	Remarks
			Total	Free	Total	Free		Total	Free						
grams			mgm. per 100 cc.		mgm. per 100 cc.		cubic centimeters	grams		cc.			mil lions per c.mm.	per cent	
2 grams Solu-dagenan intra-muscularly	May 7	5:30 p.m.	0	0							101.5	17,200	5,70	95	N.P.N., 28
		6:00 p.m.	2.1	1.9											Neutrophils 85 per cent
		7:30 p.m.	3.7	3.3											Right lower lobe involved
2 grams Solu-dagenan intra-muscularly		8:30 p.m.	4.2	4.0							102.6				No soreness at site of injection
		10:30 p.m.	6.6	5.6											
2 grams Solu-dagenan intra-muscularly	May 8	12:01 a.m.	6.2	5.2							101.2				
		9:00 a.m.	8.6	7.4	235	150	140 (S.G. = 1034)	.33	.21	590	98.4	12,000	4,71	85	
1 gram Solu-dagenan intra-muscularly		9:00 p.m.	8.2	6.7	242	167	700	1.69	1.17	2,200	101.0				No local or general reaction to injections
		10:00 p.m.	9.7	7.5											
		12:02 a.m.	9.8	7.9							100.8				
1 gram Solu-dagenan intra-muscularly	May 9	9:00 a.m.	6.0	5.2	333	211	300	1.00	0.63		101.4				Extension to left lower lobe in spite of levels in blood
		11:20 a.m.	6.7	5.8							103.4	8,900		88	
2 grams Dagenan per Os.		12:30 p.m.	6.6	5.9	231	124	200	0.46	0.25						
		2:30 p.m.	7.8	6.6											Very ill
2 grams Dagenan per Os.		4:30 p.m.	8.0	7.4						2,160	103.0				
2 grams Dagenan per Os.		6:00 p.m.	12.1	11.0							101.0	6,200			
2 grams Dagenan per Os.		11:45 p.m.	14.4	12.9							101.4				
2 grams Dagenan per Os.	May 10	4:00 a.m.									101.2				Much improved
2 grams Dagenan per Os.		8:00 a.m.									99.0	4,800	4.62	93	
1½ grams Dagenan per Os.		12:30 p.m.			288	170	1,050	3.02	1.79		98.8				

the absorption, distribution and excretion of the drug in the human subject.

2 Tentative conclusions have been drawn from the findings in 88 cases which received Dagenan by mouth.

3 The results of chemical studies on 4 cases treated with chemotherapy by injection have been compared with the conclusions reached in the orally treated cases

4 The findings in both groups of cases have been compared and discussed

The authors wish to record their thanks to Dr H K. Detweiler for his helpful suggestions in the preparation of this paper and to Miss M Dolan for valuable technical assistance.

BIBLIOGRAPHY

- 1 Evans, G M. and Galsford W F., Treatment of pneumonia with 2 (p-aminobenzene)sulphonamido) pyridine. *Lancet* 1938 2 14
- 2 Marshall E. K. Jr., Determination of sulfanilamide in blood and urine. *J Biol. Chem.*, 1937, 122, 263
- Marshall, E. K. Jr., and Litchfield, J T., Jr., Determination of sulfanilamide. *Science*, 1938 88 85
- 3 Marshall, E. K., Jr., and Litchfield J T Jr., Some aspects of the pharmacology of sulfapyridine. Read before the American Association of Physicians, Atlantic City May 2, 1939
- 4 O'Brien E. J., and McCarthy C J Staphylococcal septicaemia treated with M. & B 693 *Lancet*, 1938, 2, 1232.
- Maxwell J., Staphylococcal septicaemia treated with M & B 693 *Lancet* 1938 2, 1233
- 5 Peters, John P., Distribution and movement of water and solutes in human body *Yale J Biol. and Med.*, 1933 5 431
- 6 Stewart, J D., Rourke G M and Allen, J G., Excretion of sulfanilamide. *J A M A.*, 1938 110, 1885
- 7 Stewart, J D., Rourke, G M., and Allen, J G., Acetylation of sulfanilamide. *Surgery*, 1939 5 232.

chemical estimations to ensure effective dosage and it greatly obscures any attempt at estimating the true toxicity of the drug (3).

From the findings in this series it seems safe to state that when a single dose of sulphapyridine is given by mouth the maximum concentration in the blood is reached in four to five hours. When given intramuscularly as sodium sulphapyridine or Solu-dagenan the maximum is attained in three to three and one-half hours. When this salt is injected intravenously there is for a short period a very high concentration in the blood but this falls after thirty minutes to a level which may be taken as the effective maximum concentration. This is not observed in cases treated orally or by intramuscular injection, for, in these, distribution is effected as the drug is taken into the general circulation.

Postmortem assays of the organs, tissues and fluids indicate a selective and unequal distribution, but, in general, arbitrary calculations based on the concentration of the drug in the blood and the weight of the patient enable one to estimate with remarkable accuracy the actual amount of the drug contained in the body at a given moment.

There has been a general tendency to be content with estimating the concentration of the free moiety in the blood as a guide to treatment. The findings in the series indicate that the procedure may fall far short of the desiderata for intelligent and safe therapy. The extraordinary increase in the highly toxic conjugated fraction which has been observed in cases with temporarily defective renal function constitutes a real danger in treatment. It has been demonstrated that the impairment of the kidneys may occur several days after the pneumonic process has apparently been conquered and at times when the blood concentrations of free sulphapyridine are not excessively high.

In previous years it was noticed that the use of sulphanilamide in the treatment of Type III pneumonia not infrequently resulted in an accumulation of the drug in the blood. As much as 30 mgm per 100 cc were found in cases receiving less than 100 grains of sulphanilamide per diem. Excessively high concentrations only occurred, however, at the height of the febrile period and, in the absence of additional evidence to the contrary, they were attributed to temporary renal failure produced by the disease process.

The occurrence of acute renal insufficiency in recovering and afebrile cases on maintenance doses of sulphapyridine seems, on the other hand, to incriminate the drug itself. Some of the cases in the series developed sudden oliguria when the fluid intake was as much as 2,000 cc in twenty-four hours. Nevertheless, acute renal insufficiency with accumulation of sulphapyridine in the blood never occurred without warning. A review of the protocols of such cases always revealed that on one or more previous occasions the urine volume had fallen below 750 cc in a twenty-four-hour period. These facts force the conclusion that an accurate measurement of the urine passed in each twenty-four hours of treatment by chemotherapy is of paramount importance. At the first indication of defective renal function (urinary excretion falling below 1,000 cc in twenty-four hours), fluids must be administered in large amounts and, if the concentration for the total drug in the blood is high, chemotherapy should at once be interrupted or terminated altogether.

With the existence or restoration of normal renal function, intravenous administration results in a very rapid excretion of the drug and a prompt decrease in the concentration in the blood. This should always be the first and it is probably the only effective method of combatting serious toxic manifestations resulting from this type of chemotherapy.

In this series the evidence seems to support the view that the conjugated form is excreted in the urine at a faster rate than is the free form. Although the kidneys are undoubtedly of chief importance in removing the drug from the body, it has been shown in cases receiving the drug by injection only that considerable amounts may be excreted into the bowel.

We are not able to state how much of the drug can be lost by perspiration or by the bowel but at least in cases suffering from pneumonia it would seem to be certain that other avenues of excretion in addition to the kidneys must be in active use.

SUMMARY

1. Data accumulated in a series of 90 cases of pneumonia treated by 2-(p-aminobenzenesulphonamido)-pyridine, M & B 693 (Dagenan or sulphapyridine), have been applied to a study of

THE COAGULATION DEFECT IN HEMOPHILIA STUDIES OF THE CLOT PROMOTING ACTIVITY ASSOCIATED WITH PLASMA EUGLOBULIN IN HEMOPHILIA¹

By EUGENE L. LOZNER AND F. H. L. TAYLOR

(From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard) of the Boston City Hospital and the Department of Medicine Harvard Medical School Boston)

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Normal human cellular free citrated plasma² contains a substance which acts to decrease the coagulation time of the blood in hemophilia (1, 2). The observation of Patek and Taylor (3) that this activity is associated or co-precipitated with certain fractions of the plasma proteins has recently been confirmed by Howell (2). Both Howell's experiences and those in this laboratory would indicate that the clot promoting power is associated with the globulin portion of the plasma proteins, whether prepared by salting out (2), acid precipitation (2, 3) or dialysis (3).

During the past two years a study of the nature of "globulin substance" prepared by dilution and acid precipitation of plasma has been made (3, 4, 5). It was determined that the protein precipitates obtained between pH 5.5 and 6.0 are associated with the largest proportion of clot promoting activity. The active factor contained in such precipitates was found to be thermolabile, soluble in isotonic salt solution, and capable of being passed through a Berkefeld filter. It is rapidly destroyed by alkali and by prolonged standing in neutral solution. Solutions of "globulin substance" behave in a quantitative manner with respect to their ability to accelerate clot formation of hemophilic blood *in vitro*. Single injections of this acid precipitated "globulin substance" into patients with hemophilia result in a prompt fall in the co-

agulation time of their circulating blood. Injections of such solutions repeated every six hours however, produce a transitory refractory phase during which the coagulation time returns to the former prolonged values. At the height of the refractory period when injections of solutions of "globulin substance" are no longer effective, an injection of whole Berkefelded plasma shortens the coagulation time. It was suggested that a possible explanation of this phenomenon might lie in the presence of a factor in normal plasma which was lost in the acid precipitation of the "globulin substance." The dilution of the plasma necessary to precipitate "globulin substance" effectively by acid precluded a satisfactory investigation of this hypothesis. Thus the globulin fraction was prepared by dialysis which allowed a partition of the plasma that could be more easily investigated. In comparison with the acid precipitated material the globulin prepared by dialysis has the advantages of freedom from foreign reagents, maximum solubility in isotonic saline, and closer physico-chemical definition as "euglobulin." Euglobulin is used here in the commonly accepted sense as that portion of the plasma proteins which is insoluble in distilled water but soluble in isotonic saline. The present communication presents certain observations on the euglobulin of the blood plasma with particular respect to its clot promoting power for hemophilic blood both *in vitro* and *in vivo* and indicates certain differences existing between such preparations and those obtained by acid precipitation.

METHODS

Measured amounts of fresh Berkefelded citrated normal human plasma were pipetted into cellophane tubes and dialyzed against running tap water for eight days. The temperature of the water remained between 4° and 6° C. As dialy-

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² Hereafter the word "plasma" refers to plasma prepared from fresh normal human whole blood to which sodium citrate was added to make the final citrate concentration 0.25 per cent. This was then centrifuged 30 minutes at 2000 r.p.m., filtered through two thicknesses of Number 2 Whatman paper and passed through a Berkefeld V filter.

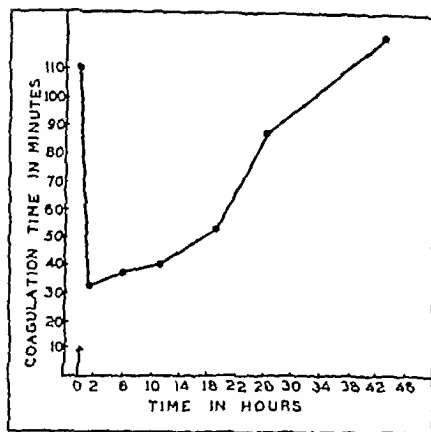


FIG. 1 THE EFFECT OF A SINGLE INTRAVENOUS INJECTION (ARROW) OF PLASMA EUGLOBULIN ON THE BLOOD COAGULATION TIME OF A PATIENT WITH HEMOPHILIA

The effect of multiple intravenous injections of plasma euglobulin on the coagulation time of the blood of patients with hemophilia. Observations concerning multiple intravenous injections of normal plasma euglobulin were made on three patients with hemophilia having blood coagulation times of 32, 105, and 139 minutes respectively. In each case, 50 ml of the saline solution of euglobulin derived from 75 ml of normal human plasma were injected intravenously at 6-hour intervals for varying periods of time. The results obtained in one patient are described here and summarized graphically in Figure 2. The results obtained in the other two patients were entirely similar. Following the first injection of plasma euglobulin, the coagulation time was found to have fallen in one hour from 139 to 23 minutes. Five hours after this injection the coagulation time had risen to 42 minutes but was brought back to 27 minutes by a second injection of 50 ml of euglobulin substance. The injections were repeated every 6 hours until 42 hours after the first injection. In each instance the patient responded with a fall in the coagulation time of his blood. Thirteen hours after the last injection the coagulation time had risen to 77 minutes and in 26 hours had returned to a level of 122 minutes, at which time observations were discontinued. There was at no time any indication of a

refractory period such as occurs accompanying multiple injections of acid precipitated "globulin substance" (4, 5). The reader is invited to compare Figure 2 of this communication with Figures 1, 2 and 3 of reference 5.

The effect of a single intravenous injection of supernatant substances from normal human plasma on the coagulation time of the blood in hemophilia. Fifty ml of the water solution of the supernatant substances derived from 75 ml of plasma were injected intravenously into a patient with hemophilia. The coagulation time of the patient's blood fell from 44 minutes to 38 minutes in the course of one hour and was 40 minutes five hours later. The difference in these figures is of no significance as these coagulation times are well within the range of the variations recorded for coagulation time in this patient on previous occasions. The coagulation time of the blood of this same patient on another occasion was sharply reduced from 32 minutes to 15 minutes following the injection of 50 ml of the saline solution of the euglobulin derived from 75 ml of the same parent plasma. The administration of the supernatant substances in the case of a second patient failed to produce any fall in the coagulation time of his blood when the material was injected intravenously six hours after an effective injection of plasma euglobulin.

DISCUSSION

The present investigations indicate that the clot-promoting power of normal human plasma for hemophilic blood is associated with the plasma euglobulin. Plasma euglobulin preparations are active in reducing the coagulation time of hemophilic blood both *in vivo* and *in vitro*. In the latter instance, their action appears to be quantitative. The coagulating activity of the supernatant substances containing the water soluble non-dialyzable portion of the plasma, however, is comparatively very slight. These findings are confirmatory of previous experiences in this laboratory and also of those of Howell who finds that most of the clot-promoting activity of plasma is associated with euglobulin and fibrinogen when such substances are prepared from plasma by salting-out methods. Howell has also shown that the clot promoting substance is not fibrinogen, since coagulating activity remains in his preparation after the removal

sis proceeded, a precipitate of euglobulin separated. When dialysis was complete, the contents of the tube were washed into 250 ml centrifuge cups and spun for 15 minutes at 2000 r p m. The supernatant liquid containing those plasma substances which were water soluble and non-dialyzable was decanted and the precipitate washed twice with distilled water.³ The washings were discarded. The plasma euglobulin and the supernatant substances were then dried by the "lyophile" process. To facilitate injections, the euglobulin from each 75 ml of blood plasma was redissolved in 50 ml of saline and an equivalent quantity of the supernatant substances was redissolved in 50 ml of distilled water. In order to avoid deterioration, an amount of the dry material sufficient to carry out each observation was made up into solution immediately before use. The solutions of the plasma euglobulin and the supernatant substances were then passed through a Berkefeld filter and transferred under sterile conditions to rubber-capped vials and kept in the ice box until the time of injection. Three separate preparations of plasma euglobulin were investigated. For each preparation 600 ml of fresh-pooled normal human plasma were used. The methods of injection of the material, and the determination of coagulation times, and of the activity of various preparations to be studied have been fully described elsewhere (4).

EXPERIMENTAL

The clot-promoting activity in vitro of plasma euglobulin and supernatant substances. To varying amounts of the saline solution of plasma euglobulin and water solution of the supernatant substances, 2 ml of blood from a patient with hemophilia were added according to the standard technique previously described (2). Each of the three preparations was tested on blood from at least two patients. One dilution, 0.05 ml of the solutions to be tested to 2 ml of hemophilic blood, only, was used of the third preparation. The results are given in Table I. It will be observed that the saline solution of plasma euglobulin was optimally active and behaved in a quantitative man-

³ Hereafter these substances will be referred to as supernatant substances and plasma euglobulin respectively.

TABLE I

The clot-promoting activity for hemophilic blood in vitro of plasma euglobulin and supernatant substances

Preparation number	Patient number	Ml added to 2 ml hemophilic blood	Saline solution of plasma euglobulin Coagulation time	Water solution of supernatant substances Coagulation time
1 Jan 17, 1939	1	milliliters 0 (control) 01 05 10	minutes 172 73 12 11	minutes 172 142 53 42
1	2	0 (control) 01 05 10	32 20 14 10	32 32 25 15
2 Feb 6, 1939	2	0 (control) 01 05 10	45 16 9 5	45 49 27 27
2	3	0 (control) 01 05 10	139 20 9 7	139 113 40 22
3 Feb 24, 1939	2	0 (control) 05	44 12	44 40
3	3	0 (control) 05	110 10	110 29
3	4	0 (control) 05	65 11	65 37
3	5	0 (control) 05	106 12	106 40

ner in reducing the coagulation time of hemophilic blood. The solution of the supernatant substances however, contained only small amounts of activity.

The effect of a single intravenous injection of plasma euglobulin on the coagulation time of the blood of a patient with hemophilia. A patient with hemophilia was given a single intravenous injection of 50 ml of the saline suspension of the euglobulin derived from 75 ml of normal human plasma. The coagulation time fell abruptly from 110 minutes to 32 minutes in the course of the first hour after injection and remained below 40 minutes for 11 hours, after which it slowly returned to pre-injection levels, reaching a value of 120 minutes 43 hours after injection. The results are given in graphic form in Figure 1. These results are in every way comparable to those reported for the effect of the injection of Berkefeld normal human plasma (1).

2. Howell W H., Hemophilia. Bull. New York Acad. Med. 1939 15, 3
3. Patek A J., Jr and Taylor, F H L., Hemophilia some properties of substance obtained from normal human plasma effective in accelerating coagulation of hemophilic blood J Clin. Invest. 1937 16 113
4. Pohle, F J., and Taylor F H L., Coagulation defect in hemophilia Effect in hemophilia of intramuscular administration of globulin substance derived from normal human plasma. J Clin. Invest., 1937, 16, 741
5. Pohle, F J and Taylor F H L., Coagulation defect in hemophilia. Studies on refractory phase following repeated injections of globulin substance derived from normal human plasma in hemophilia. J Clin. Invest., 1938 17 779
6. Lomer E. L., Kark, R., and Taylor F H. L., The coagulation defect in hemophilia. The clot promoting activity in hemophilia of Berkeley'd normal human plasma free from fibrinogen and prothrombin. J Clin. Invest., 1939, 18 603

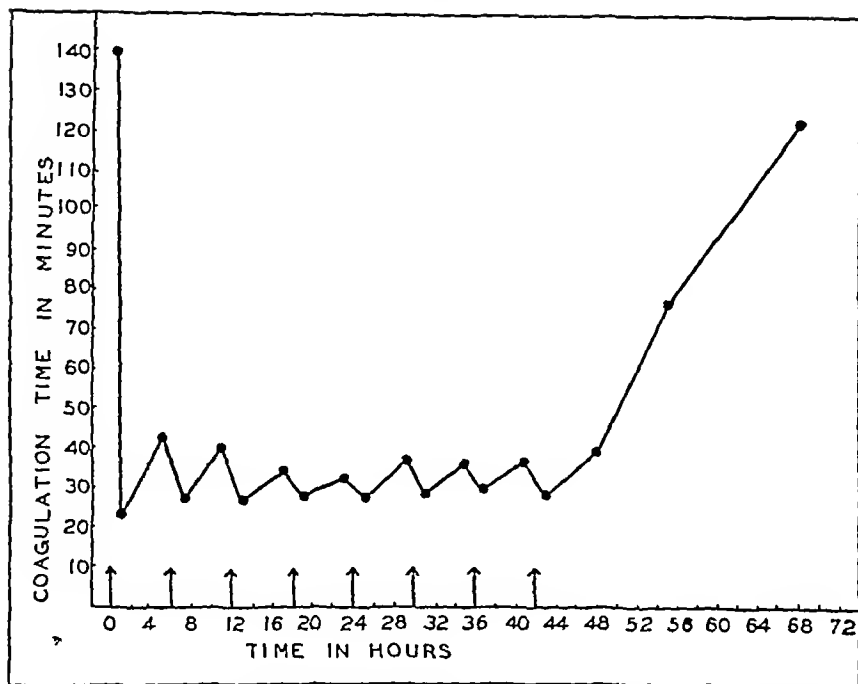


FIG 2 THE EFFECT OF MULTIPLE INTRAVENOUS INJECTIONS (ARROWS) OF PLASMA EUGLOBULIN ON THE BLOOD COAGULATION TIME OF A PATIENT WITH HEMOPHILIA

of this protein. It has recently been shown (6) that plasma freed from both fibrinogen and prothrombin possesses clot-promoting activity. It appears evident, therefore, that while a clot-promoting substance is to be found in close association with the fibrinogen and euglobulin of the blood plasma, its identity with any given protein contained therein cannot be assumed. However, it may well be in combination with these proteins or co-precipitated with them.

The difference between the behavior of plasma euglobulin observed here and that previously observed for acid-precipitated "globulin substance" was quite unexpected. Single injections of both plasma euglobulin and "globulin substance" into hemophilic patients produce a prompt decrease of blood coagulation time. The duration of the effect following the injection of plasma euglobulin seems to be greater than that previously described for "globulin substance." In all other respects, single injections of these substances behave similarly and resemble the effect of an injection of the parent plasma from which they are derived. Repeated injections of plasma euglobulin, in sharp contrast to those of "globulin substance," behave

in a comparable manner to injections of whole plasma, in that they are capable of maintaining the coagulation time at the lowered level with no indication of a refractory period which develops after injections of "globulin substance." It would seem, therefore, that plasma euglobulin presented a more reasonable starting point for further fractionation than that offered by acid-precipitated "globulin substance."

SUMMARY AND CONCLUSIONS

1 Dialysis of cellular-free citrated normal human plasma yields a euglobulin precipitate containing practically all of the clot-promoting activity of the plasma for hemophilic blood.

2 Unlike acid-precipitated "globulin substance," plasma euglobulin resembles normal human plasma in its ability to maintain in hemophilia a reduced level of the blood coagulation time when injected intravenously every six hours.

BIBLIOGRAPHY

- 1 Patek, A. J., Jr., and Stetson, R. P., Hemophilia, abnormal coagulation of blood and its relation to blood platelets. *J. Clin. Invest.*, 1936, 15, 531.

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ERRATUM

On page 484 of the July 1939 issue in the last sentence of the summary of an article on *Crystalline Insulin* by Dr Alexander Marble and Dr Ilmari Vartiainen the word *amorphous* should have read *crystalline*